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# The effect of excess trace minerals on pig performance and dietary tocopherols

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# The effect of excess trace minerals on pig performance and dietary tocopherols

by

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# GENERAL LITERATURE REVIEW

## Introduction

Osborne and Mendel (1919) and Mattill and Conklin (1920) reported reproductive failure in rats fed semi-purified diets supplemented with all known required nutrients. The presence of a fat soluble dietary factor X in lettuce, wheat germ and alfalfa meal that corrected the reproductive failure was first reported by Evans and Bishop (1922). The factor was designated vitamin E (E being next in the alphabetical series) by Sure (1924) and Evans (1925).

Evans et al. (1936) isolated two compounds from wheat germ oil that had vitamin E activity. These were named alpha- and beta-tocopherol. The term tocopherol was derived from the Greek terms tocos, meaning offspring, and phero, meaning to bear. The ol ending was used to identify the compound as an alcohol. Emerson et al. (1937) isolated a third biologically active tocopherol from cottonseed oil which was designated gamma-tocopherol. Delta-tocopherol was isolated from soybean oil (Stern et al., 1947). The identification of four tocotrienols from vegetable oils as a separate group of compounds was reported in the mid 1960s (Pennock et al., 1964; Whittle et al., 1966). The study of vitamin E was further complicated by the discovery of the essentiality of the mineral, selenium, as an anti-necrogenic factor that could prevent some symptoms of vitamin E deficiency (Schwarz and Folz, 1957).

## Chemistry and Biological Activity

Structure and nomenclature. The structure of alpha-tocopherol was first reported correctly by Fernholz (1938) as a result of degradation studies. The tocopherols are substituted tocols (Nelis et al., 1985). The tocopherols that occur in nature differ by the number and position of the methyl groups on the chromanol ring. Alpha-tocopherol has methyl groups at positions 5, 7, and 8. Beta-tocopherol has methyl groups at positions 5 and 8. Gamma-tocopherol has methyl groups at positions 7 and 8. Delta-tocopherol has a methyl group at position 8. The tocotrienols have three double bonds in the isoprene side chain and have similar differences in the number and positions of the methyl groups on the chromanol ring (McCay and King, 1980).

Tocopherols have chiral centers at C2, C4' and C8'. The natural compounds each have an all trans configuration (RRR). However, the synthetically produced tocopherols from natural phytol produce a mixture of epimers differing in configuration at the C2 position and tocopherols from racemic phytol produce a mixture of all eight diastereo-isomers (Diplock, 1985).

Consequently, there has been some confusion in the naming of the various compounds. Natural RRR-alpha-tocopherol is referred to as *d*-alpha-tocopherol as a result of the observation that it rotated polarized light to the right in an ethanol solution. This method of naming is considered

unsatisfactory as the rotation of polarized light by RRR-alpha-tocopherol is solvent dependent. The synthetic ester of alpha-tocopherol containing all 8 diastereo-isomers has been referred to as *dl*-alpha-tocopheryl acetate (Diplock, 1985).

In 1974, the IUPAC-IUB Commission on Biochemical Nomenclature recommended that natural alpha-tocopherol be designated as RRR-alpha-tocopherol. The commission also recommended that alpha-tocopherol synthesized from natural phytol be designated as 2-ambo-alpha-tocopherol, and that alpha-tocopherol synthesized from synthetic phytol or isophytol be designated as all-rac-alpha-tocopherol. The remaining tocopherol isomers are named in the same manner with beta, gamma or delta being substituted for alpha. The tocotrienols are named under the same convention as the tocopherols with the term tocotrienol substituted for tocopherol. The commission also recommended that vitamin E be used as a generic term describing all of the tocopherol and tocotrienol compounds exhibiting the biological activity associated with RRR-alpha-tocopherol (IUPAC-IUB Com. On Biochem. Nomenclature, 1974). The IUPAC-IUB commission recommendations on naming tocopherols are currently used in scientific literature, while the older, trivial names continue to appear in the popular press.

Bioassays. Several bioassays have been used to evaluate the biological activity of the tocopherol and tocotrienol

isomers. The bioassays are based on several different biological responses and can be categorized into three types depending on the physiological response involved. These are: a) bioassays measuring a biological function such as prevention of fetal resorption in rats; b) measurement of a physiological parameter such as the prevention of erythrocyte hemolysis; and c) measurement of vitamin E levels in vivo such as liver storage (Ames, 1971).

The bioassays measuring a biological function are the oldest and best documented measures of the biological activity of tocopherols. The fetal resorption assay in rats is the most widely used and was the original assay that led to the discovery of vitamin E (Diplock, 1985; Evans and Bishop, 1922). In the rat fetal resorption assay, female rats that are deficient in vitamin E are test mated with normal males. Upon being bred, the females are then fed a diet containing a known amount of a tocopherol standard or the test material. The females are then killed on the nineteenth day of pregnancy, and examined for the presence of live embryo and implantation sites. A female with one or more live embryos is considered positive, while animals with no live embryos are considered negative. Thus, this assay is an all or nothing response assay. Animals with less than four implantation sites are removed from the experimental results. The biopotency of the test compound is the ratio of the amount of the test compound required to supply a 50% fertility dose

compared to the amount of the standard required to supply a 50% fertility dose (Mason and Harris, 1947; Desai, 1980; Bliss and Gyorgy, 1967).

Another bioassay that measures biological function is the prevention of encephalomalacia in chicks. This assay also works on the all or nothing principle with an animal considered negative if symptoms are exhibited (Ames, 1971). Positive and negative in this assay appear to refer to the ability of the test ingredient to prevent deficiency symptoms.

The prevention of erythrocyte hemolysis in the presence of dialuric acid is the major assay measuring a physiological response. The hemolysis assay measures the ability of rat erythrocytes to remain intact in the presence of dialuric acid (Rose and Gyorgy, 1925; Friedman et al., 1958; Bliss and Gyorgy, 1967) or hydrogen peroxide (Nitowsky et al., 1956; Gyorgy et al., 1952). The change in the percentage of cells that are hemolyzed before vs 40-44 hours after the dose is used as a measure of vitamin E activity. Other hemolysis assays measuring spontaneous hemolysis in an isotonic saline solution (Draper and Csallany, 1969) or hemolysis in the presence of a glucose-glucose oxidase peroxide system (Barker et al., 1973) have been reported.

Muscular dystrophy has been used as a physiological index for several species including rats (Filer et al., 1946), rabbits (Hove and Harris, 1947), chicks (Scott and

Desai, 1964) and ducklings (Jager and Verbeek-Raad, 1970). The dystrophic lesions and creatinuria are measured on a scale and the scale score of the test compound is compared to the scale developed for the standard (Desai, 1980; Diplock, 1985). The accuracy of physiological assays to determine biopotency has been questioned as the assay measures only what can be absorbed into the system of the animal and does not take into account dietary or environmental factors (Diplock, 1985; Hove and Harris, 1947)

Measurement of in vivo tocopherol levels gives a good indication of the current status of the animal; however, like the physiological indexes the in vivo assay is affected by dietary and environmental factors as well as the species and tissue being assayed. The chick liver storage assay is probably the most widely used assay of this type. In the chick liver storage assay and all assays of this type, the animal is fed a deficient diet prior to the start of the test. The animal is then switched to a diet containing a standard or the test compound. The animals are killed after a specified time and the tissue level is measured with the test compound being compared to the standard (Bliss and Gyorgy, 1967; Pudelkiewicz et al., 1960).

Biological activity. The biological activity of vitamin E is expressed in international units. The international unit (IU) of vitamin E was established in 1947 and defined as 1.0 mg of 2-ambo-alpha-tocopheryl acetate and was the average

amount of the vitamin needed to prevent fetal resorption in rats when given as an oral dose (Diplock, 1985). The original standard was produced by Hoffman-La Roche Inc., Nutley, NJ., from natural phytol. The ratio of the two stereo isomers in the standard compound was never determined. Consequently, duplicate batches of the original standard were never made. Currently tocopheryl acetate is synthesized from synthetic phytol, and therefore there is no standard compound remaining to directly determine biological activity in terms of the original IU (Diplock, 1985; Bieri and McKenna, 1981).

In 1957, the World Health Organization (WHO), the agency that had originally defined the IU for vitamin E, reported that the IU for vitamin E had ceased to exist. The IU has continued to be used in labeling and commercial purposes. "However, such use of the Unit does not distinguish between the various stereo-isomers of alpha-tocopherol and the 'International Unit' has come to mean 1.0 mg of alpha-tocopherol regardless of its nature" (Diplock, 1985).

The WHO in 1982 discussed the need for a reference unit for vitamin E and agreed that such a unit would be useful, but could not agree if a biological or chemically based unit would be the most helpful. Finally it was decided that since the molecular and stereochemical composition of the tocopherols could be determined, that it would be inappropriate to designate a fixed biological reference unit and that the therapeutic activity of the individual

tocopherols could be evaluated from clinical evidence. The WHO left it to the individual nations to assess the therapeutic claims for individual tocopherols (Diplock, 1985; WHO Tech. Report 687, 1983).

The American Pharmaceutical Association (APA) has recommended a weight unit relationship for some of the widely used tocopherols. These values are shown in table 1 (The National Formulary XI, 1960).

Table 1. The APA weight/unit relationship of tocopherol

| Tocopherol | units/mg |
|---|---|
| All-Rac-alpha-tocopheryl acetate | 1.0 |
| All-Rac-alpha-tocopherol | 1.1 |
| All-Rac-alpha-tocopheryl succinate | 0.89 |
| RRR-alpha-tocopherol | 1.49 |
| RRR-alpha-tocopheryl acetate | 1.36 |
| RRR-alpha-tocopheryl succinate | 1.21 |

Ames (1979) determined the biopotencies of several forms of alpha-tocopherol in the rat fetal resorption assay over a period of 21 years. The tocopherols were compared to the original IU standard 2-ambo-alpha-tocopheryl acetate. The results indicate that RRR-alpha-tocopheryl acetate has a relative biopotency of 1.66, which is significantly higher

than the 1.36 used by the APA. The values reported by Ames (1979) for RRR-alpha-tocopheryl succinate and all-rac-alpha-tocopheryl acetate were significantly lower than the APA values. Ames felt that these data supported using RRR-alpha-tocopheryl acetate as a standard rather than all-rac-alpha-tocopheryl acetate.

The relative biopotency of the various tocopherols tends to be the same regardless of which bioassay is used. Values reported for RRR-alpha-tocopheryl acetate range from 1.66 in the rat fetal resorption assay to 1.47 in the hemolysis assay and 1.34 in the chick tissue storage assay (Ames, 1979; Pudelkiewicz et al., 1960; Friedman et al., 1958). The biopotency of other natural forms when compared to the natural alpha-tocopherol (100) are beta-tocopherol 15-49, gamma-tocopherol 3-19, delta-tocopherol <1, alpha-tocotrienol 17-21 and beta-tocotrienol 1-4 (Brubacher and Wiss, 1972).

Chemical assays. The classical method for the determination of tocopherols is the colorimetric method of Emmerie and Engel (1938). This method utilized the ability of tocopherols to reduce $Fe^{+3}$ to $Fe^{+2}$ in the presence of ethanol. The ferrous ions then reacted with 2,2'-dipyridyl to form a red complex that was measured at 540 nm. The different tocopherols reacted variably in the assay and the sensitivity was impaired by high blank values. The method was greatly improved by Tsen (1961) when

4,7-diphenyl-1,10-phenanthroline was used in place of 2,2'-dipyridyl.

The spectrophotometric assay has found limited use in the quantitation of tocopherols. Tocopherols exhibit a low intensity of absorption at the low end of the ultraviolet scale (292 nm). The usefulness of the spectrophotometric method is limited to relatively pure samples containing relatively high concentrations of tocopherols (Bunnell, 1967).

Spectrofluorometric methods were found to be more sensitive than colorimetric methods. Originally tocopherols were reacted with fluorometric compounds (Kofler, 1942). However, it was soon discovered that all tocopherols possessed a natural fluorescence (Duggan, 1959). Several of the common high performance liquid chromatography (HPLC) methods in use today utilize spectrofluorometers as detectors. These methods report tocopherol values that are very repeatable with recoveries of 70-90% (Taylor et al., 1976; Cort et al., 1983; Cohen and Lapointe, 1980). It should be noted that exposure to ultraviolet light destroys the tocopherols (Diplock, 1985).

Recently, electrochemical detection in conjunction with HPLC has been reported as an alternative way to measure tocopherol content in blood and tissues. This method has a high sensitivity with low baseline noise and good reproducibility (Lang et al., 1986). The major drawback to the method

appears to be the expense of the equipment required for detection.

## Metabolism

Absorption and excretion. In rats, the absorption of tocopherols is dependent on fat absorption and the presence of bile and pancreatic lipase. Tocopherol esters are hydrolyzed in the intestine and tocopherols are absorbed, as alcohols, in the small intestine. The free alcohols enter the intestinal lacteals and are transported via the lymph to the general circulation. In the rat, tocopherols in the plasma are generally attached to the globulin fraction of the lipoproteins. The major route of excretion of the tocopherol seems to be in the bile as free tocopherol (Ullrey, 1981).

Bile and pancreatic juice both seem to be required for the absorption of tocopherol into the lymph. Gallo-Torres (1970, 1973) reported that in rats, 10% of an orally administered dose of radioactively labeled alpha-tocopheryl acetate was present in the lymph 12 hours after administration with normal bile and pancreatic juice excretion. When either the bile duct or the pancreatic duct was diverted, only negligible amounts of alpha-tocopherol appeared in the lymph. Absorption of alpha-tocopheryl acetate returned to normal when both ducts were restored to normal (Gallo-Torres, 1970, 1973). The hydrolysis of alpha-tocopheryl acetate is dependent on the presence of bile and pancreatic fluid, and the

detergent properties of the bile salts aid in the formation of tocopherols into mixed micelles with other lipids (Diplock, 1985).

The intestinal environment affects the absorption of tocopherols. Studies indicate that tocopherols are absorbed better from an aqueous environment than an oily environment (Schmandke and Schmidt, 1965). The presence of mono-olein or triolein stimulates tocopherol absorption, while the presence of linoleic acid depresses the absorption of tocopherol (Gallo-Torres, 1973; Akerib and Steiner, 1971).

The major route of tocopherol excretion is through the feces. Feces contain tocopherol secreted from intestinal cells, desquamation of intestinal epithelia, incomplete absorption and bile (Diplock, 1985). Klaskin and Molander (1952) found 64.4% of the calculated daily tocopherol intake of man was excreted in the feces. Rabbits were also reported to excrete 65-80% of their daily tocopherol intake in the feces (Simon et al., 1956).

There is some controversy as to the source of the biliary tocopherol. Schmandke and Proll (1964) reported that tocopherol was secreted unchanged in the bile, while MacMahon et al. (1971) and Gallo-Torres (1980) found less than 2% of a radioactive dose of tocopherol was excreted in the bile, with a majority of the radioactivity representing a compound thought to be a glucuronic acid conjugate of tocopherol.

Urinary excretion represents about 1% of the tocopherol excretion under normal physiological conditions. Higher urinary levels may be seen when larger amounts of tocopherol are injested (Diplock, 1985). The skin may play an important role in tocopherol excretion. The level of radioactivity is reported to be high in dermal tissue following injection of radio-labeled tocopherol (Shiratori, 1974). The chemical nature of the radioactive compounds in the dermal tissue is not known.

Tissue levels. Prior to the widespread use of HPLC methods to determine tocopherol levels, the procedures were long and difficult. Consequently there is little information regarding the normal levels of tocopherols in blood or tissue. Most of the tissue values reported in the literature are for rats. Lang et al. (1986) report alpha-tocopherol values for rat plasma, 11-13 ug/ml; rat liver, 22-35 ug/g wet wt; human blood, 6-8 ug/ml; human plasma, 9-11 ug/ml and human liver mitocondria, 43-86 ug/g protein. Taylor et al. (1976) reported alpha-tocopherol levels in rats (ug/mg protein) for liver, 1.3; heart, .12; lung, .14; and kidney, .05. In pigs, plasma tocopherol levels have been reported by Meyer et al. (1981) to be .1-.13 mg/100 ml at weaning and decreasing to .03-.07 mg/100 ml 5 weeks postweaning.

## Functions of Vitamin E

Biological antioxidant. The search for the biological function of tocopherol began shortly after the identification of tocopherol as a vitamin. Evans and Burr (1927a, b) reported that vitamin E activity was affected by fats and in particular the rancidity factors in fats. Mattill (1927) associated the absorption of oxygen and a long series of oxidation products to the development of rancidity of fats. The destruction of vitamin E activity was associated to the oxidation of fats and the catalytic properties of ferrous ions on the oxidation of fat. Tocopherols have been found to be good antioxidants in vitro and function as free radical scavengers in vivo (McCay and King, 1980; Dam, 1957).

The formation of the free radicals and the chain reaction initiated by auto-oxidation can be represented as follows (Burton and Ingold, 1986):

initiation:

$$RH + OH^- \rightarrow R^\cdot + H_2O$$

propagation:

$$R^\cdot + O_2 \rightarrow ROO^\cdot$$

$$ROO^\cdot + RH \rightarrow ROOH + R^\cdot$$

termination:

$$ROO^\cdot + ROO^\cdot \rightarrow \text{molecular products}$$

In this scheme, RH represents the oxidizable bis-allylic methylene unit of an unsaturated lipid molecule and $R^\cdot$ a free radical derived from the removal of the hydrogen atom. The

tocopherols and other antioxidants break the oxidation cascade by reacting with the ROO• molecule.

ROO• + ArOH --> ROOH +ArO•

ROO• + ArO• --> stable molecular products

ArOH represents the antioxidant compound.

The ability of alpha-tocopherol to be an effective chain breaking antioxidant is thought to be associated with the weakness of the O-H bond on carbon 6 of the chromanol ring; therefore the phenoxyl radical derived from alpha-tocopherol is thought to be quite stable (Burton and Ingold, 1986).

Burton and Ingold (1986) found the antioxidant activity of the tocopherols in an in vitro kinetic system was alpha > beta = gamma > delta. This is similar to the biological activity as determined by bioassay. This is exactly opposite of what other investigators have reported (Green et al. 1967). Burton and Ingold (1986) concluded that the difference in the antioxidant activities that they observed was due to the induction period of the assay being held constant for all of the tocopherol isomers. Alpha tocopherol was found to be a better antioxidant than the commercial antioxidant, 2,6-di-tert-butyl-4-methylphenol, in this system.

Immune response. The addition of pharmacological doses of vitamin E to animal diets has resulted in improved antibody production and host resistance to challenge (Tengerdy, 1980). Chicks fed a diet containing 150-300 mg

vitamin E as all-rac-alpha-tocopheryl acetate had an increased resistance to *E. coli* infection. Chicks on the diet supplemented with vitamin E had death losses of 0-10%, while those on the normal diet had death losses of 25-30% (Heinzerling et al., 1974). The increased resistance corresponded to elevated antibody titers, suggesting that a higher antibody level contributed to the increased resistance (Tengerdy, 1980).

Two to threefold increases in primary anti-*E. coli* serum antibodies were found in pigs fed a diet containing 110 IU/kg when compared to controls fed a nutritionally complete ration (vitamin E level not reported) not supplemented with vitamin E. Pigs fed a diet supplemented with the recommended level of vitamin E (22 IU/kg) had antibody levels intermediate to the other treatments. The secondary titers from a booster injection of the same vaccine were equal (Ellis and Vorhies, 1976).

The mechanism by which vitamin E functions in the immune system is unknown. There is speculation that vitamin E helps to maintain the membrane integrity of the lymphoid cells and thus affects the immune response mechanisms (Sheffy and Schultz, 1979). In the rat, the vitamin E requirement to maintain optimum immune responses is higher than other physiological requirements. Bendich et al. (1986) found that rats required 7.5 mg/kg vitamin E for normal growth, 15 mg/kg to prevent myopathy and 50 mg/kg to prevent red blood cell

hemolysis. The dietary requirement to maintain optimal T and B lymphocyte responses was greater than 50 mg/kg vitamin E. This suggests that immune responses may be the first indices affected by diets low in vitamin E.

Biological membranes. Biological membranes, composed primarily of phospholipid molecules, cholesterol, and membrane bound proteins, require protection from peroxidation. This appears to be one of the major functions of vitamin E in biological membranes (Burton and Ingold, 1986). Burton and Ingold (1986) reported that the chromanol ring of tocopherol was in the proximity of the carbonyl groups of the fatty acyl chains. Alpha tocopherol was believed to be quite mobile, "bobbing up and down" from an average position to react with peroxyl radicals, which were thought to have large dipole movements. The phytyl tail is believed to play an important role in the recognition of RRR-alpha-tocopherol by membranes. RRR-alpha-tocopherol is thought to be preferentially absorbed due to chiral discrimination. This may partially explain the differences in biological activity exhibited by the different stereo isomers of alpha-tocopherol (Burton and Ingold, 1986).

There is limited evidence indicating that vitamin E may have additional functions in biological membranes. Giasuddin and Diplock (1979) found that optimal growth and glucose transport by a mouse fibroblast cell line, which was developed to study biological membranes, depended on the

presence of vitamin E, linoleic acid and cholesterol. It was thought that the cholesterol was needed because of a lack of endogenous cholesterol. The antioxidant, butylated hydroxytoluene was unable to replace the vitamin E in the system. Giasuddin and Diplock (1981) found that alpha-tocopherol increased the uptake of cholesterol and cholesteryl esters in cultured cells. They found that alpha-tocopherol changed the fatty acid profiles of the membrane phospholipids, increasing the arachidonic acid content and decreasing the linoleic acid content. This affect was thought to be independent of the action of tocopherol as an antioxidant controlling the peroxidation of unsaturated fatty acids.

Vos et al. (1973) and Molernaar et al. (1972) showed in a vitamin E deficient duckling model that fatty acid composition of liver fractions was changed when vitamin E was supplemented. In these studies, arachidonic acid levels of the inner mitochondrial membrane, the outer mitochondrial membrane and the microsomal fraction were increased in the vitamin E supplemented animals. The levels of other unsaturated fatty acids tended to decrease, but none were significantly altered. The levels of the saturated fatty acids were not affected by vitamin E supplementation. Similar results were observed with a rat model (Fujita and Matsumoto, 1974) and supported by the results found in cultured cells (Giasuddin and Diplock, 1981).

## Deficiency Symptoms in Swine

Physical. One of the first reports of vitamin E deficiency symptoms in swine was by Adamstone et al. (1949), who reported a lack of coordination and decreased reproductive efficiency in sows fed semi-purified diets. Obel (1953) found vitamin E/Se deficiencies to be characterized by hepatosis dietetica (HD), a disease with no warning symptoms that caused pigs to become sluggish and die within a few hours, muscular dystrophy (MD) and fibrinoid degeneration of vascular walls. Grant (1961) demonstrated that mulberry heart disease (MHD) was a direct result of vitamin E/Se deficiency. Grant showed that tocopherol or Se would prevent MHD and HD, but MD was prevented by tocopherol only.

Ewan et al. (1969) reported that 54% of young pigs fed diets containing cod liver oil and deficient in tocopherol and Se died within 14 weeks. Pigs necropsied exhibited hepatic necrosis, icterus, general edema, anemia, stomach ulcers, pale muscle in the heart and skeleton and a yellow-brown discoloration of the adipose tissue. Histological lesions were reported in the liver and skeletal muscles. The addition of either 136 IU tocopherol or .5 ppm Se reduced mortality to 7%.

Enzymes. The activities of several blood enzymes are altered by a vitamin E/Se deficiency. None of the enzymes are organ specific and are dependent on the equilibrium between the rate of release from damaged cells and the rate

of inactivation or removal from the blood stream. The changes in enzyme activities are helpful in the diagnosis of disease and give a general indication of tissue damage (Ullrey, 1981). The activities of plasma aspartate amino--transferase (AspAT, also known as glutamic-oxalacetic transaminase [GOT]) and ornithine carbamyl transferase (OCT) are useful indicators of muscular dystrophy and liver necrosis, respectively (Ullrey, 1981). Tollersrud (1973) reported that the activities of AspAT, alanine amino-transferase (AlaAT, also known as glutamic-pyruvic transaminase [GPT]) and isocitrate dehydrogenase (ICD) increase in plasma from pigs fed semi-purified diets low in vitamin E and Se. The AspAT and ICD activity was higher in pigs receiving a diet high in protein and low in vitamin E and Se than in pigs receiving a diet low in protein, vitamin E and Se, indicating an increased liver involvement. Hepatosis dietetica was the dominate pathological diagnosis in this group of pigs. High activities of all three enzymes were thought to indicate involvement of skeletal, cardiac and hepatic tissue.

Hyldgaard-Jensen (1973) reported that changes in the activity of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) are more specific for changes in skeletal muscle. Pigs with vitamin E deficiency had large increases in the LDH activity in the serum. The increases were age dependent and the pattern of the LDH isoenzymes gave an indication of which tissues were involved.

The enzyme glutathione peroxidase (GSH-Px) is a selenium containing enzyme. Serum GSH-Px activity decreases linearly as dietary intake of Se decreases (Adkins and Ewan, 1984; Hakkarainen et al., 1978a). Serum and tissue Se levels increase as dietary Se levels increase (Mahan and Moxon, 1978).

Field deficiencies. Several factors are thought to contribute to occurrence of field deficiencies in the United States. The movement of U.S. swine producers to total confinement systems has eliminated the access to pasture and the forage crops that contain high levels of tocopherols (Ullrey, 1981). Producers in the U.S. tend to rely on corn-soybean meal diets. The tocopherol content of corn is affected by harvesting, storage and processing conditions and may be inadequate to support optimal nutrition (Young et al., 1975). Soybean meal is not expected to contain large amounts of tocopherol, because tocopherol is removed when the oil is extracted. Bunnell et al. (1968) reported that soybean meal contained from 1-5 mg/kg alpha-tocopherol and corn had 11-35 mg/kg. Young et al. (1975) found corn to contain 9.3 mg/kg, indicating that considerable variation occurs.

The use of spectrofluorometric HPLC methods to determine the alpha-tocopherol content of feed indicates that the above values are inaccurate. Cort et al. (1983) reported that the alpha-tocopherol levels in 11 corn samples ranged from 2-15 mg/kg and the alpha-tocopherol level in 15 soybean meal

samples ranged from .84-2.87 mg/kg. The method used by Bunnell et al. (1968) did not separate the alpha-tocotrienol from the alpha-tocopherol. The lower levels of alpha-tocopherol reported by Cort et al. (1983) indicate that pigs fed corn-soybean meal diets without supplementation of vitamin E may not receive the 11 mg/kg recommended by the NRC (1979). To date, there has been no explanation given for the continued occurrence of vitamin E/Se deficiency symptoms reported in pigs being supplemented with vitamin E and Se (Mahan and Moxon, 1978).

## Vitamin E Requirements for Swine

General. The vitamin E requirement of domestic animals is dependent on a number of factors and a minimum requirement can not be established independent of these factors (Gallo-Torres, 1972). In swine, the vitamin E requirement is dependent on the dietary content of selenium (Se), sulfur-amino acids and polyunsaturated fats. The stress the animals are exposed to and the availability of the vitamin E in the feed also affect the vitamin E requirement (Gallo-Torres, 1972).

The NRC (1979) recommends that swine diets contain 11 IU/kg supplemental vitamin E and .15 mg/kg Se. There is little agreement among researchers as to the adequacy of these recommendations. In Michigan, where the Se content of the soil is low, deficiencies have been reported in the field

in a large number of swine herds (Trapp et al., 1970) Unfortunately, the vitamin E levels of the diets were not reported, but the investigators felt that the vitamin E requirement for pigs was approximately 22 IU/kg (10 IU/lb) in the presence of .1 ppm Se. Other reports from Michigan (Michel et al., 1969) indicate that death losses were experienced in herds consuming a corn-soybean meal diet containing 5-8 IU/kg of vitamin E and .05 mg Se/kg. The supplementation of the diets with 22 IU/kg vitamin E prevented further losses.

Several controlled studies have been conducted to determine the dietary concentrations of vitamin E and selenium needed to prevent death losses and morphological symptoms in pigs. Ullrey (1981) summarized a number of studies involving practical swine diets, including 14 studies from the U. S. and Canada utilizing corn-soybean meal diets. From these studies it was concluded that diets which contained 5 IU/kg vitamin E and .04 mg Se/kg were not adequate for growing and finishing pigs. Supplements containing 11 IU/kg vitamin E or .1 mg Se/kg prevented mortality and deficiency symptoms and supported normal performance in growing and finishing pigs. Reproductive performance was maintained by diets containing 5-7 IU/kg vitamin E and .15 mg Se/kg, but not by diets containing 15 IU/kg vitamin E and .03 mg Se/kg. Mahan (1978) found that 13% of the pigs weaned from sows fed diets supple-

mented with 22 IU/kg vitamin E and .1 mg Se/kg died of vitamin E-Se deficiency.

Mahan et al. (1980) and Meyer at al. (1981) reported that the weanling pig required a minimum of .3 mg Se/kg when pigs were fed either 10 or 20 IU/kg of vitamin E. The selenium requirement reported was higher than the legal FDA limit at that time. Ullrey (1981) recommended that diets contain 10-20 IU/kg of vitamin E and the maximum legal amount of Se and that producers with problem herds should consider adding up to 30/kg IU of vitamin E to their diets.

Interaction with selenium. Vitamin E and glutathione peroxidase, a selenium containing enzyme, play important roles in the biological antioxidant system. Selenium, as sodium selenite, has been reported to be 500 times more active than vitamin E in the effective prevention of necrotic liver degeneration (Schwarz and Foltz, 1957). In rats, a deficiency or large excess of vitamin E depresses GSH-Px levels in tissues and serum. When rats were fed diets supplemented with from 25 to 25,000 IU of all-rac-tocopheryl acetate/kg of diet, the activity of GSH-Px was decreased linearly ($r = -0.82$). Glutathione peroxidase activity was also depressed in rats receiving diets containing no supplemental tocopherol (Yang and Desai, 1978). Thompson and Scott (1969) found that the vitamin E content of the diet affected the Se requirement and the Se requirement of chicks increased as the dietary vitamin E level decreased. There is also some

evidence that excess vitamin E increased the urinary excretion of Se in swine (Groce et al., 1973)

Semi-purified diets containing 5 IU of vitamin E and .135 mg Se/kg were found to effectively reduce mortality and prevent deficiency symptoms, but did not maintain adipose tissue tocopherol levels. A minimum vitamin E concentration of 15 IU/kg was needed to maintain tocopherol levels in adipose tissue (Hakkarainen et al., 1978b). Bengtsson et al. (1978) found that the addition of 45 mg alpha-tocopherol/kg prevented vitamin E/selenium deficiency symptoms, but did not prevent selenium depletion of the plasma when pigs were fed a diet containing .008 mg Se/kg. Orstadius et al. (1963) found that vitamin E and Se were more effective in reducing the severity of MD in pigs when fed together than when fed separately. Scott (1965) reported that Se and tocopherol fed together were more effective in treating MD in chicks than either alone. Riker and Wedam (1963) found similar results in sheep and cattle.

Ewan et al. (1969), Ewan and Wastell 1970) and Wastell et al. (1972) fed torula yeast or soy protein diets containing cod liver oil to 2-3 week old and growing and finishing pigs. The diets contained .04-.06 ppm Se and were supplemented with 0 or .5 ppm Se and 0 or 136 IU vitamin E. The vitamin E level of the basal diets was not reported. In these experiments, the addition of Se or vitamin E alone was sufficient to prevent vitamin E-Se deficiency symptoms and no

additional response was shown when vitamin E and Se were fed together. It was noted that only tocopherol supplementation prevented the increases in enzyme activity associated with tissue degeneration.

Interactions with fat. Many of the early studies on vitamin E used diets containing fat as a source of the other fat soluble vitamins. Evans and Burr (1927a, b) and Mattill (1927) reported experiments where vitamin E deficiencies could be induced faster when rats were fed diets containing lard in place of butter or wheat germ oil. Mattill and Golumbic (1942) stated that the confusion in the literature on the ability of various fats to induce muscular dystrophy could be reconciled on the basis of the disappearance of the vitamin E either before of after ingestion of the ration. The rate of disappearance was dependent on the character and rate of oxidation of the accompanying unsaturated fats. One of the functions of tocopherols is as an antioxidant. Tocopherols perform this function in the feed as well as in the body. Consequently, animals fed diets with high polyunsaturated fat levels require additional vitamin E. The amount of additional vitamin E required depends on the source of the fat, the degree of unsaturation and the storage conditions of the feed (Chow and Draper, 1974).

Effects of processing. There has been considerable research reported on the effects of processing grains and forages on the tocopherol levels. Tocopherol levels are

decreased during processing by light, heat, alkali, exposure to trace elements and oxidation. The tocopherols can be protected by chelating agents or antioxidants, such as ascorbic acid (Diplock, 1985). Crops dried in sunlight have lower levels of tocopherols than crops dried in the shade. The tocopherol levels of crops dried artificially are influenced by the amount of heat used to dry the crop and the length of time the crop is dried. The losses of tocopherols during storage depend on the storage temperature and the moisture content of the feed (Kivimae and Carpena, 1973).

Young et al. (1975) found that the method of storage and drying had dramatic effects on the tocopherol levels in corn. Corn harvested at 75% dry matter (DM), with a tocopherol content of 9.3 mg/kg DM, was stored in several ways. Corn that was artificially dried, reaching a maximum temperature of 68 C, had a tocopherol content of 9.0 mg/kg DM 230 days later. The tocopherol levels of corn spread on trays and dried in the dark at 21 C decreased linearly to levels of 5.3 mg/kg DM after 48 days and changed very little to 230 days. The peroxide values of the naturally dried corn increased dramatically during the first 48 days and then slowly returned to normal. The high peroxide values are thought to account for the high rate of tocopherol destruction during the first 48 days of the experiment. Corn that was treated with propionic acid or a 60:40 mixture of acetic:propionic acid at a rate of 1-1.25% by weight and stored at ambient

temperature had tocopherol levels of 1.2 mg/kg DM after 230 days.

Richter et al. (1982) reported that vitamin E levels in concentrates decreased 4% over a one year period and that vitamin E concentrations in mixed diets decreased 25% over the same period. Tocopherol concentrations were further reduced by increased storage temperatures and the presence of trace minerals. The trace minerals used or the amounts present were not reported. Young et al. (1975) found that alpha-tocopheryl acetate was stable in diets containing high moisture corn for up to 84 days.

The effect of airtight storage on the vitamin E content of high moisture barley is variable depending on storage conditions and moisture content of the barley. Hakkarainen et al. (1983a, b) found the total vitamin E content of barley containing 13% moisture and stored under normal storage conditions decreased from 80 mg/kg to 70 mg/kg in an 11 month period, a loss equal to about 1% per month. The vitamin E content of barley containing 23% moisture, stored in an airtight silo, decreased from 92 mg/kg to 20 mg/kg during the 11 month experiment. The vitamin E levels of the barley in silos fitted with an expansion sack or external $CO_2$ supply decreased 3% per month to a level of 66 mg/kg.

Barley harvested at 28% moisture had a lower vitamin E content than the 23% or 13% moisture barley throughout the entire experiment. The vitamin E content of the 28% moisture

barley was decreased from 80 mg/kg to 10-15 mg/kg. The addition of an expansion sack to the silo also protected the vitamin E in the 28% barley. High moisture barley had an increased rate of vitamin E oxidation (Hakkarainen et al. (1983b).

Interactions with trace minerals. The effects of metal cations on the tocopherol levels in mixed livestock diets have not been thoroughly investigated. Mattill (1927) found that ferrous ions stimulated the oxidative destruction of vitamin A and E. Waddell and Steenbock (1931) found that the addition of 1% ferric chloride to the standard ration would produce sterility in rats faster and more consistently than other vitamin E deficient diets known at the time.

Tollerz and Lannek (1964) pretreated pigs with vitamin E, Se or ethoxyquin and were able to prevent the mortalities associated with iron dextran injection. Baby pigs from sows fed a vitamin E/Se deficient diet injected with iron dextran to prevent baby pig anemia have an increased mortality rate (Patterson and Allen, 1972). Miller et al. (1973) and Loudenslager et al. (1986), however, were unable to produce an iron intolerance in pigs from sows fed corn-soybean meal diets unsupplemented with vitamin E or Se. Pigs from sows fed 5% aerated cod liver oil or pigs dosed with 5-10 ml of aerated cod liver oil developed myopathy identical to vitamin E/Se deficiency when injected with 750 mg of iron dextran (Ullrey, 1981).

In rats, there are conflicting reports regarding the effectiveness of vitamin E in preventing iron induced auto-oxidation of polyunsaturated fats. Dougherty et al. (1981) found that an injection of 30 mg/kg ferrous chloride increased the production of ethane, a volatile autoxidation product of omega-3-unsaturated fatty acids, 8 fold in rats fed a vitamin E deficient diet compared to rats fed a diet supplemented with 200 IU/kg vitamin E.

Iron dextran injected at 500 mg Fe/kg was fatal to rats fed a diet deficient in vitamin E and Se or a diet supplemented with .5 ppm Se. The addition of 200 IU vitamin E/kg to the diet prevented mortality. Iron dextran injections increased ethane production 4 fold in rats fed the vitamin E/Se deficient diet and 3 fold in the diet supplemented with Se. Supplementation with vitamin E prevented the rise in ethane production associated with the injection of iron dextran (Dougherty et al., 1981). Diplock et al. (1967) using smaller doses of iron (50 mg Fe/kg rat) in the presence of 1000 IU/kg vitamin A and .1 mg/kg alpha-tocopherol were unable to show an increase in the rate of oxidation of polyunsaturated fats fed to the rats and concluded that Fe overloading had no effect on the auto-oxidation of fats in the rat.

Lindvall et al. (1980), while treating an elderly man for anemia resulting from malabsorption disease, produced disturbances in the cardiac rhythm after 5 injections with

iron-sorbitol and a persistent complete heart block following the seventh injection. The patient was found to have extremely low serum tocopherol levels and after a series of tocopherol injections (amount not reported) the intolerance to iron disappeared.

Copper is added to swine diets, normally as copper sulfate, to promote growth. The response to the addition of copper to swine diets is variable (NRC-42, 1974). Several investigators have reported improved performance (Barber et al., 1968; Kline et al., 1972), while Amer and Elliot (1973) and Parris and McDonald (1969) reported no response to the addition of 250 ppm copper. The presence of copper in swine diets has been found to change the physical and chemical properties of depot fat. Decreases in the melting point of the depot fat have been reported in pigs receiving diets supplemented with 250 ppm copper. The decrease in melting point is attributed to an increased proportion of unsaturated fatty acids (Amer and Elliot, 1973) and an alteration in the structure of the fat triglycerides (Christie and Moore, 1970).

Supplemental vitamin E has been shown to reduce the susceptibility of the depot fat to oxidation, hypothetically by being deposited in fat and serving as an antioxidant (Amer and Elliot, 1973). The use of tocopherol as an antioxidant to control the susceptibility of pork fat to rancidity was found to be impractical. Watts et al. (1946) found that

feeding tocopherol in natural rations provided little protection to the fat, and that tocopherol injections provided even less protection from rancidity.

High dietary levels of copper can cause selenium deficiencies and prevent selenium toxicity. Jensen (1975) fed chicks diets containing 800 or 1600 ppm copper and observed a high incidence of exudative diathesis and muscular dystrophy when the diets were not supplemented with Se. The addition of 0.5 ppm Se prevented all deficiency symptoms. The addition of Se did not prevent the growth depression normally associated with excess dietary copper. Rats fed a diet containing 200 ppm copper and .06 ppm Se had decreased GSH-Px activity in the blood, liver, testes and kidney and decreased tissue selenium levels when compared to controls fed .06 ppm Se (Rahim et al., 1986).

High levels of dietary zinc can also cause a selenium deficiency. Chicks fed diets containing 2100 or 4100 ppm zinc and .2 ppm Se developed a high incidence of exudative diathesis and muscular dystrophy. Zinc is also believed to interfere with the selenium utilization in much the same way as copper does. However, the exact mechanism of the interference was not determined in these experiments (Jensen, 1975).

Several other minerals have been found to affect the vitamin/Se status of animals. The addition of water soluble silver salts increased the incidence and severity of vitamin

E deficiencies in rats and chicks and the addition of selenium to the diet prevents the deficiency symptoms (Shaver and Mason, 1951; Bunyan et al., 1968; Peterson and Jensen, 1975). Cadmium was found to decrease selenium absorption and reduce glutathione peroxidase activity (Flegal et al., (1980). The toxic effects of mercury and cadmium can be counteracted by the supplementation of the diet with Se (Ganther et al., 1972; Parizek and Ostadalova, 1967).

## Conclusions

Since the discovery of vitamin E in 1922, many researchers have studied the various aspects of the vitamin. The structure and biological activities of the tocopherol isomers have been established. Deficiency symptoms have been identified for most domestic animals and man. The tocopherols have been found to have several functions in the body. The exact biochemical reactions of most of these functions have yet to be elucidated. The interaction of tocopherols with other dietary factors, notably polyunsaturated fats, selenium and other trace minerals, has made the study of tocopherols extremely complex.

Several studies in swine have determined the level of tocopherol and Se needed for optimal performance (Ullrey, 1981). Pigs in commercial units being fed levels of tocopherols and Se at or above the recommendations continue to exhibit symptoms of vitamin E/Se deficiency. There is

limited evidence that the tocopherol and/or selenium recommendations for swine are too low (Mahan, 1978; Mahan et al., 1980).

The effect of storage environment and diet composition on the stability of tocopherols has been established (Chow and Draper, 1974; Young et al. 1975). Studies at Iowa State University have indicated that swine diets in Iowa tend to contain high levels of trace minerals, particularly iron, zinc, and copper (Ewan, 1986). The effect of high levels of trace minerals in swine diets on the performance of pigs or the stability of tocopherols in mixed feeds is unknown.

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## EXPLANATION OF DISSERTATION FORMAT

The following sections are written as papers for submission to the Journal of Animal Science and follow the style and form of the Journal of Animal Science. The research reported was conducted by Charles Robert Dove under the supervision of Dr. R. C. Ewan. Mr. Dove was responsible for the completion of all aspects of the experiments described.

# SECTION 1. THE EFFECT OF EXCESS COPPER, IRON OR ZINC ADDITION ON THE TOCOPHEROL AND SELENIUM STATUS OF GROWING PIGS


## Abstract

Two experiments were conducted to evaluate the effect of the addition of high levels of copper, iron or zinc to swine starter and grower diets on pig performance and vitamin E-selenium status. The stability of natural tocopherols in mixed feeds was evaluated during storage. Addition of 1000 ppm iron or 1000 ppm zinc had no affect on performance in either experiment. In Exp. 1, the addition of 250 ppm copper improved gains and feed efficiency ($P<.05$) during the first three weeks of the experiment. The advantage was lost during week four and no other effect was seen on performance in either experiment as result of the addition of copper. Serum lactic dehydrogenase (LDH) or glutathione peroxidase (GSH-Px) activity was not affected by any of the mineral additions. Serum tocopherols were not affected by the mineral treatments in Exp. 1. In Exp. 2, addition of 250 ppm Cu decreased ($P<.05$) serum tocopherol levels throughout the entire 8 week experiment. The alpha-tocopherol levels of the starter diet decreased ($P<.05$) during storage. Addition of 250 ppm Cu ($P<.01$) or 1000 ppm Fe ($P<.05$) or 1000 ppm Zn ($P<.05$) increased the destruction of alpha-tocopherol in the starter diet. Alpha- and gamma-tocopherol levels of the starter diet

decreased to near zero in 22 days in the presence of 250 ppm copper. In the grower diet, the addition of 250 ppm Cu ($P<.01$) or 1000 ppm Fe ($P<.05$) increased the destruction of tocopherols. The addition of 1000 ppm Zn had no affect ($P>.1$) on tocopherol destruction in the grower diet. The addition of high levels of trace minerals had no affect on the performance or serum enzymes of growing pigs, but the addition of copper decreased serum tocopherols.

Key Words: Swine, Tocopherol, Iron, Zinc, Copper, Selenium.

## Introduction

The effect of the moisture content of swine diets on tocopherol levels during storage and the development of vitamin E/Se deficiencies has been well established (Young et al., 1977, 1975; Sharp et al., 1972). Other factors that contribute to the vitamin E/Se requirement of pigs include environmental stress, polyunsaturated fat content of the diet and infection (Najman et al., 1976; Naftalin and Howie, 1969; Keahey and Whitehair, 1966).

Recent studies have found that swine diets in Iowa tend to contain high levels of Fe, Cu and Zn (Ewan, 1986). The effects of high levels of trace minerals on tocopherols has not been thoroughly investigated. These studies were conducted to determine the effect of high levels of dietary trace minerals on the performance and the tocopherol and

selenium status of growing pigs. The effect of trace minerals on the natural tocopherols in the feed during storage was also evaluated.

## Materials and Methods

Experiment 1. Thirty-six crossbred pigs (Yorkshire X Landrace X Duroc, average initial weight of 6.0 kg at five weeks of age) were assigned from litter outcome groups to one of six treatments. Two levels of copper, from anhydrous cupric sulfate, (5 or 250 ppm) and three levels of iron, from ferrous sulfate·$7H_2O$, (100, 500 or 1000 ppm) were used in a 2 X 3 factorial arrangement of treatments.

The pigs were weaned and penned by litter for six days prior to beginning the 8-wk experiment. Pigs were penned individually during the first four weeks of the experiment and 2/pen during the remainder of the experiment. Pigs had ad libitum access to feed and water. Pigs were weighed and feed consumption was determined weekly. Weekly serum samples were stored at -20 C until analyzed for tocopherols and the activity of lactic dehydrogenase (LDH) and glutathione peroxidase (GSH-Px). Serum enzyme activities were determined within 24 hours after blood samples were collected.

The starter diet was fed during the first four weeks and the grower diet was fed during the remainder of the experiment (table 1). Diets were formulated to meet or exceed all NRC (1979) recommendations except for vitamin E

and selenium. No supplemental vitamin E or selenium was added to the diets.

Selenium was analyzed by the method of Olson et al. (1975). Serum LDH was determined by the ultra-violet spectrophotometric method of Amador et al. (1963). Serum GSH-Px was determined by the ultra-violet spectrophotometric method of Paglia and Valentine (1967). Serum was extracted with HPLC grade hexane and analyzed for tocopherols utilizing the HPLC method of Cort et al. (1983) with fluorometric determination. The mobile phase was 3.5% tetrahydrofuran (v/v) in hexane with a flow rate of 2.0 ml/minute.

Experiment 2. Sixty-four crossbred pigs (Yorkshire X Landrace X Duroc, average initial weight 5.8 kg) were assig~ed from litter outcome groups to one of eight treatments for the eight week experiment. Two levels of Cu (5 or 250 ppm) from $CuSO_4$, two levels of Fe (100 or 1000 ppm) from $FeSO_4 \cdot 7H_2O$ and two levels of Zn (100 or 1000 ppm) from ZnO were utilized in a 2 X 2 X 2 factorial arrangement of treatments. Pigs were placed on experiment immediately after weaning. Pigs were penned and data were collected as described for Exp. 1. The starter diet was fed the first four weeks of the experiemnt and the grower diet the remainder of the experiment (table 1).

Feed samples were collected weekly and stored at -20 C until they were analyzed for tocopherols. Feed samples were ground through a 1.0 mm screen in a centrifugal force

grinder. Five gram samples were extracted in acetone for 2 to 3 hours, evaporated to dryness, redissolved in 10 ml of HPLC grade hexane, filtered through a .8 micron membrane filter and injected into the HPLC system. Chromatographic conditions and detection were the same as described for serum tocopherols.

Statistics. Least Squares analysis of variance was used for Exp. 1 (Harvey, 1966). The general linear model procedure of SAS (1982) was used for Exp. 2.

## Results and Discussion

Performance. There were no interactions observed for any of the measurements taken; therefore, only the data for the main effects are reported. Average daily gain (ADG) and average daily feed intake (ADF) increased ($P<.05$) and gain:feed decreased ($P<.05$) as the animals grew older (tables 2 and 3). Performance was similar in both experiments. There were no significant ($P>.1$) effects on performance observed as a result of the iron or zinc treatments. The addition of copper increased ($P<.05$) ADG during the first three weeks of Exp. 1 (table 2). Copper had no effect on performance during the remainder of Exp. 1 or in Exp. 2. The effects of copper supplementation were variable and similar to results reported previously (NRC-42, 1974).

Serum enzymes. Serum LDH activity decreased ($P<.05$) the first week of both experiments and then remained relatively

stable (tables 4 and 5). Elevation of serum LDH is an indicator of nutritional muscular dystrophy (NMD, Paulson et al., 1968), associated with vitamin E-Se deficiencies (Hartley and Grant, 1961). LDH activity has been shown to be increased in pigs fed Vitamin E/Se deficient diets (Ewan and Wastell, 1970). The relative stability of the LDH activity in these experiments indicates that no vitamin E-Se deficiency was present.

Activity of GSH-Px was not affected ($P>.1$) in either experiment by any of the mineral treatments (tables 4 and 5). GSH-Px is a Se containing enzyme that has antioxidant functions (Tappel, 1984). The GSH-Px activity was higher in Exp. 2 than Exp. 1 and may indicate that the pigs used in Exp. 2 maintained a better Se status (Adkins and Ewan, 1984). The dietary Se levels were similar in both experiments (table 1). Very high levels of Cu (800 ppm) and Zn (2100 ppm) have been shown to induce Se deficiency symptoms in chicks not supplemented with Se (Jensen, 1975). No evidence of vitamin E-Se deficiency was observed in any of the pigs used in these experiments.

Serum Tocopherols. Serum alpha-tocopherol levels in Exp. 2 decreased ($P<.05$) the first week of the experiment (table 5). The serum tocopherol levels of pigs in both experiments remained low throughout the entire experimental period. The decrease in serum tocopherol was not seen during the first week of Exp. 1 (table 4), because pigs had been

weaned 6 days prior to the start of the experiment. Meyer et al. (1981) reported that plasma tocopherol levels decreased the first week postweaning and remained lower than weaning levels for the first 5 weeks postweaning. Mahan and Moxon (1980) found that nursing piglets had high plasma tocopherol levels which decreased following weaning in pigs not receiving supplemental vitamin E. Loudenslager et al. (1986) reported that the serum tocopherol levels of pigs from dams not supplemented with vitamin E might decrease faster than the serum tocopherol levels of pigs from supplemented dams. It would appear that natural feed ingredients do not contain adequate natural tocopherols or that young pigs are unable to absorb adequate amounts of the natural tocopherols to maintain serum tocopherols at preweaning levels.

Serum tocopherol levels in Exp. 2 were further decreased ($P<.05$) the first 4 weeks of the experiment by the addition of 250 ppm Cu when compared to the 5 ppm Cu diet (figure 1). The serum tocopherol levels of pigs receiving 250 ppm Cu began to return to normal following the diet change at the end of week 4, but remained lower than the pigs fed 5 ppm Cu through out the entire experiment. Meyer et al. (1981) found that plasma tocopherol levels increased linearly as feed tocopherol increased.

Feed Tocopherols. The alpha-tocopherol level of all starter diets decreased ($P<.05$) during storage (table 6). The addition of 1000 ppm Fe or Zn to the starter diet

increased (P<.05) the destruction of both alpha- (table 6) and gamma-tocopherol (data not shown). The destruction of alpha- and gamma-tocopherol was increased (P<.01) by the addition of 250 ppm Cu to the starter diet (table 6) and tocopherol levels decreased to near zero after 22 days of storage in the diet containing 250 ppm Cu.

The addition of 250 ppm Cu (P<.01) or 1000 ppm Fe (P<.05) increased the destruction of both alpha- (table 6) and gamma-tocopherol (data not shown) in the grower diet. Addition of 1000 ppm Zn to the grower diet had no affect (P>.1) on tocopherol destruction.

The addition of soybean oil caused the tocopherol levels of the starter diets to be higher than the tocopherol levels of the grower diets. The increased destruction of the natural tocopherols appears to be a result of the soybean oil addition to the starter diet. The decrease in the tocopherol levels of the diets containing 250 ppm Cu are reflected in the serum tocopherol levels, especially during the first 4 weeks of the experiment. These experiments suggest that the addition of Cu at growth promoting levels (250 ppm) increases the loss of the tocopherols in the feed. Low levels of feed tocopherols in diets containing 250 ppm Cu had no affect on performance or serum enzymes, but did decrease serum tocopherols. Supplemental tocopherol may, therefore, be required to maintain serum tocopherol levels in pigs receiving 250 ppm Cu.

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Table 1. Composition of experimental diets

| Ingredient | Experiment 1 Starter | Experiment 1 Grower | Experiment 2 Starter | Experiment 2 Grower |
|---|---|---|---|---|
| | % | | | |
| Corn, ground yellow | 57.3 | 76.7 | 48.6 | 70.9 |
| Soybean meal, 48% | 22.3 | 19.1 | 29.0 | 24.6 |
| Whey | 15.0 | | 15.0 | |
| Soybean oil, crude | 1.0 | | 2.9 | |
| Dicalcium phosphate | 1.1 | 1.3 | 1.0 | 1.1 |
| Calcium carbonate | .7 | .9 | .9 | .9 |
| Iodized salt | .25 | .25 | .25 | .25 |
| Antibiotic [a] | .25 | .25 | .25 | .25 |
| DL-methionine | .1 | | | |
| Mineral mix [b] | 1.0 | 1.0 | 1.0 | 1.0 |
| Vitamin mix [c] | 1.0 | .5 | 1.0 | 1.0 |
| Analyzed composition | | | | |
| Crude protein (%) | 19.0 | 17.5 | 20.4 | 18.2 |
| Gross energy (kcal/kg) | 3951 | 3896 | 4129 | 3950 |
| Selenium (ppm) | .43 | .50 | .41 | .42 |
| Copper (ppm) | | | | |
| 5 ppm diet | 12 | 12 | 10 | 11 |
| 250 ppm added diet | 255 | 256 | 243 | 247 |
| Iron | | | | |
| 100 ppm diet | 263 | 296 | 247 | 304 |
| 500 ppm added diet | 659 | 696 | | |
| 1000 ppm added diet | 1165 | 1223 | 1122 | 1238 |
| Zinc | | | | |
| 100 ppm diet | 128 | 129 | 112 | 104 |
| 1000 ppm added diet | | | 950 | 955 |
| Calculated composition | | | | |
| Lysine (%) | .90 | .69 | 1.20 | .95 |

[a]Contributed per kg of diet: 110 mg sulfamethazine, 55 mg penicillin and 110 mg chlortetracycline.

[b]Contributed per kg of diet: 30 ppm Mn, 1.5 ppm I, 5 or 250 ppm Cu, 100, 500 or 1000 ppm Fe and 100 or 1000 ppm Zn.

[c]Contributed per kg of diet: 4400 IU vitamin A palmitate, 1100 IU vitamin $D_2$, 6.6 mg riboflavin, 17.6 mg d-pantothenic acid, 33 mg niacin and 22 ug vitamin $B_{12}$.

Table 2. Least squares means of the performance of growing pigs fed varying levels of copper and iron (Exp. 1)

| Week | Cu, ppm | | Fe, ppm | | |
|---|---|---|---|---|---|
| | 5 | 250 | 100 | 500 | 1000 |
| | Average Daily Gain, Kg | | | | |
| 1 | .13 | .21[a] | .16 | .13 | .12 |
| 2 | .35 | .44[a] | .40 | .40 | .38 |
| 3 | .37 | .47[a] | .44 | .40 | .43 |
| 4 | .55 | .47 | .50 | .55 | .49 |
| 1-4 | .35 | .40 | .37 | .37 | .38 |
| 5-8 | .54 | .57 | .56 | .56 | .55 |
| | Average Daily Feed, Kg | | | | |
| 1 | .25 | .30 | .25 | .24 | .23 |
| 2 | .52 | .61 | .55 | .54 | .60 |
| 3 | .68 | .80 | .75 | .71 | .75 |
| 4 | .89 | .89 | .88 | .92 | .87 |
| 1-4 | .58 | .65 | .61 | .60 | .64 |
| 5-8 | 1.22 | 1.23 | 1.24 | 1.22 | 1.22 |
| | Gain:Feed | | | | |
| 1 | .62 | .65 | .73 | .58 | .62 |
| 2 | .68 | .71 | .73 | .74 | .63 |
| 3 | .54 | .58 | .57 | .55 | .56 |
| 4 | .61 | .50 | .56 | .57 | .51 |
| 1-4 | .61 | .60 | .64 | .60 | .57 |
| 5-8 | .44 | .43 | .42 | .43 | .47 |

[a]Effect of Cu level ($P<.05$).

Table 3. Least squares means of the performance of growing pigs fed varying levels of copper, iron and zinc (Exp. 2)

| Weeks | Cu, ppm | | Fe, ppm | | Zn, ppm | |
|---|---|---|---|---|---|---|
| | 5 | 250 | 100 | 1000 | 100 | 1000 |
| | Average Daily Gain, Kg | | | | | |
| 1-4 | .31 | .33 | .32 | .32 | .31 | .33 |
| 5-8 | .62 | .66 | .65 | .64 | .64 | .65 |
| | Average Daily Feed, Kg | | | | | |
| 1-4 | .47 | .48 | .47 | .48 | .47 | .48 |
| 5-8 | 1.39 | 1.44 | 1.40 | 1.42 | 1.42 | 1.41 |
| | Gain:Feed | | | | | |
| 1-4 | .66 | .69 | .68 | .67 | .66 | .69 |
| 5-8 | .45 | .46 | .46 | .45 | .45 | .46 |

Table 4. Least squares means of the serum enzyme activities and tocopherol levels of growing pigs fed diets containing varying levels of copper or iron (Exp. 1)

| Week | Cu, ppm | | Fe, ppm | | |
|---|---|---|---|---|---|
| | 5 | 250 | 100 | 500 | 1000 |
| | GSH-Px, units/ml[a] | | | | |
| Initial | .82 | .86 | .84 | .84 | .84 |
| Week 1 | .82 | .68 | .79 | .76 | .70 |
| Week 4 | .77 | .82 | .77 | .74 | .88 |
| Week 8 | .71 | .68 | .71 | .66 | .71 |
| | LDH, units/ml[b] | | | | |
| Initial | 642 | 586 | 664 | 597 | 582 |
| Week 1 | 533 | 498 | 554 | 499 | 494 |
| Week 4 | 356 | 302 | 333 | 327 | 327 |
| Week 8 | 368 | 381 | 378 | 378 | 366 |
| | Alpha-tocopherol, mcg/ml | | | | |
| Initial | .28 | .30 | .32 | .30 | .25 |
| Week 1 | .29 | .19 | .30 | .22 | .19 |
| Week 4 | .41 | .47 | .44 | .46 | .42 |
| Week 8 | .41 | .41 | .39 | .43 | .39 |

[a]One unit was defined as the amount of enzyme that will convert one umol NADPH per minute at 20 C and pH 7.0 (Paglia and Valentine, 1967).

[b]One unit was defined as the amount of enzyme per ml of serum that will decrease optical density 0.001 unit $\cdot$ $minute^{-1}$ $\cdot$ $centimeter^{-1}$ of light path at 340 mu and 20 C (Amador et al., 1963).

Table 5. Least squares means of the serum enzyme activities and tocopherol levels of growing pigs fed diets containing varying levels of copper, iron of zinc (Exp. 2)

| Weeks | Cu, ppm | | Fe, ppm | | Zn, ppm | |
|---|---|---|---|---|---|---|
| | 5 | 250 | 100 | 1000 | 100 | 1000 |
| | GSH-Px, units/ml[a] | | | | | |
| Initial | .77 | .74 | .75 | .76 | .73 | .78 |
| Week 1 | 1.15 | 1.05 | 1.05 | 1.14 | 1.13 | 1.07 |
| Week 4 | .94 | .86 | .87 | .94 | .91 | .89 |
| Week 8 | 1.19 | 1.21 | 1.18 | 1.22 | 1.20 | 1.20 |
| | LDH, units/ml[b] | | | | | |
| Initial | 510 | 514 | 512 | 512 | 523 | 500 |
| Week 1 | 362 | 346 | 349 | 360 | 343 | 366 |
| Week 4 | 477 | 456 | 461 | 474 | 468 | 466 |
| Week 8 | 386 | 414 | 406 | 394 | 417 | 382 |
| | Alpha-tocopherol, mcg/ml | | | | | |
| Initial | .90 | .88 | .83 | .95 | .88 | .90 |
| Week 1 | .45 | .30[c] | .35 | .41 | .44 | .31 |
| Week 4 | .29 | .08[c] | .20 | .18 | .18 | .20 |
| Week 8 | .23 | .16[c] | .18 | .20 | .20 | .20 |

[a]One unit was defined as the amount of enzyme that will convert one umol NADPH per minute at 20 C and pH 7.0 (Paglia and Valentine, 1967).

[b]One unit was defined as the amount of enzyme per ml of serum that will decrease optical density 0.001 unit $\cdot$ $minute^{-1}$ $\cdot$ $centimeter^{-1}$ of light path at 340 mu and 20 C (Amador et al., 1963).

[c]Effect of Cu level ($P<.05$).

Table 6. Least squares means of alpha-tocopherol levels of diets containing varying levels of copper, iron or zinc during storage, mcg/g (exp. 2)

| Days Stored | N | Cu, ppm | | Fe, ppm | | Zn, ppm | |
|---|---|---|---|---|---|---|---|
| | | 5 | 250 | 100 | 1000 | 100 | 1000 |
| | ---------- Starter Diet[a] ---------- | | | | | | |
| 1 | 8 | 5.04 | 3.88[b] | 4.51 | 4.40[c] | 4.52 | 4.40[d] |
| 8 | 8 | 4.46 | 1.45 | 2.77 | 3.13 | 3.27 | 2.64 |
| 15 | 8 | 4.07 | 0.05 | 2.26 | 1.86 | 2.16 | 1.96 |
| 22 | 8 | 3.00 | 0.01 | 1.73 | 1.28 | 1.57 | 1.43 |
| 29 | 8 | 2.85 | 0.00 | 1.76 | 1.06 | 1.57 | 1.28 |
| | ---------- Grower Diet ---------- | | | | | | |
| 0 | 12 | 4.22 | 3.27[b] | 3.88 | 3.64[c] | 3.96 | 3.54 |
| 1 | 4 | 3.93 | 3.31 | 3.71 | 3.52 | 3.73 | 3.49 |
| 3 | 12 | 4.23 | 2.44 | 3.50 | 3.17 | 3.34 | 3.33 |
| 8 | 4 | 4.17 | 3.23 | 3.80 | 3.61 | 3.56 | 3.83 |
| 10 | 12 | 4.05 | 0.92 | 2.77 | 2.20 | 2.41 | 2.56 |
| 15 | 4 | 4.64 | 2.27 | 4.02 | 2.89 | 3.30 | 3.61 |
| 17 | 12 | 3.70 | 0.30 | 2.24 | 1.76 | 2.21 | 1.88 |

[a]Alpha-tocopherol levels decreased in all diets due to storage ($P<.05$).

[b]Effect of Cu level ($P<.01$).

[c]Effect of Fe level ($P<.05$).

[d]Effect of Zn level ($P<.05$)

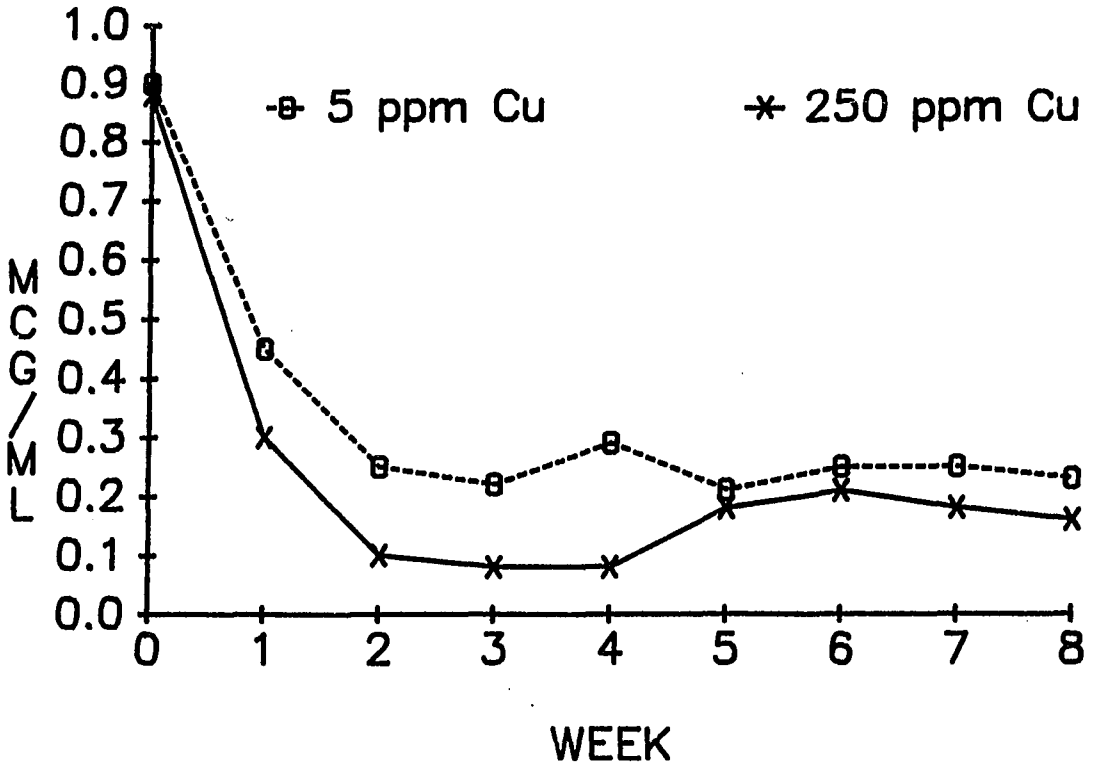


Figure 1. The effect of cooper addition on serum alpha-tocopherol levels (Exp 2). Decrease from initial levels during week 1 ($P<.01$). Effects of levels of Cu during weeks 1 to 4 ($P<.01$) and during weeks 5 to 8 ($P<.05$)

# SECTION 2. THE EFFECT OF EXCESS TRACE MINERALS ON THE STABILITY OF VITAMIN E IN SWINE GROWER DIETS


## Abstract

The stability of alpha-tocopheryl acetate and natural tocopherols in swine grower diets containing high levels of trace minerals was studied in a 12 week experiment. Corn-soybean meal diets with either 0 or 1% crude soybean oil (SBO) added were supplemented with no trace minerals (NOTM), a standard trace mineral mix (TM), TM + 250 ppm copper (Cu), TM + 1000 ppm iron (Fe), TM + 1000 ppm zinc (Zn) or TM + 100 ppm manganese (Mn). Alpha-tocopheryl acetate levels in the NOTM diet were stable during the 12 weeks of storage. The addition of TM, Cu, Zn or Mn had no effect ($P>.1$) on the alpha-tocopheryl acetate levels. The addition of Fe resulted in an increased ($P<.05$) rate of alpha-tocopheryl acetate destruction. The addition of SBO had no effect ($P>.1$) on the rate of alpha-tocopheryl acetate destruction. The alpha-tocopherol levels of the NOTM diet decreased ($P<.01$) to about 50% during the 12 week storage period. The addition of TM had no effect ($P>.1$) on the rate of alpha-tocopherol destruction. The addition of Cu ($P<.001$), Fe ($P<.01$), Zn ($P<.01$) or Mn ($P<.05$) resulted in an increased rate of alpha-tocopherol destruction. The addition of Cu decreased ($P<.01$) alpha-tocopherol levels to near 0 in 12 days. The addition of SBO to the diet containing TM ($P<.01$), Fe

(P<.01), Zn (P<.01) or Mn (P<.05) further increased the rate of alpha-tocopherol destruction. The destruction of natural gamma tocopherol was similar to alpha-tocopherol. This experiment indicates that the rate of oxidation of natural tocopherols and alpha-tocopheryl acetate is increased in the presence of certain trace minerals.

Key words: Swine, Tocopherol, Copper, Iron, Zinc, Manganese, Storage.

## Introduction

The levels of natural tocopherols present in feed ingredients vary with environmental conditions. Young et al. (1975) reported a higher rate of natural tocopherol oxidation in high moisture corn due to increased peroxidation of the corn. Hakkarainen et al. (1983a, b) reported similar results in high moisture barley and found that storage of barley under anaerobic conditions decreased the rate of tocopherol oxidation in high moisture barley.

The addition of excess levels of trace minerals to swine diets seems to be a common practice among swine producers (Ewan, 1986). Richter et al. (1982) found that the addition of excess levels of a trace mineral mix to a swine diet increased the rate of tocopherol destruction. Dove and Ewan (1987) reported that copper, iron and zinc increased natural tocopherol oxidation during storage, however the stability of alpha-tocopheryl acetate was not studied. This experiment

was conducted to determine the stability of alpha-tocopheryl acetate and tocopherols in swine grower diets in the presence of excess copper, iron, zinc or manganese during storage.

## Materials and Methods

A corn-soybean meal swine grower diet containing 18% crude protein with 0 or 1% crude soybean oil substituted for corn (table 1) was studied. Vitamins were added to meet NRC 1979 recommendations, except for tocopherol which was added at 20 IU/kg as D,L-alpha-tocopheryl acetate[1]. The diets were supplemented with no added trace minerals (NOTM), used as the control, a standard trace mineral mix (TM), TM + 250 ppm copper (Cu) added as $CuSO_4$, TM + 1000 ppm iron (Fe) added as $FeSO_4 \cdot 7H_2O$, TM + 1000 ppm zinc (Zn) as ZnO and TM + 100 ppm manganese (Mn) as $MnSO_4 \cdot 7H_2O$. Replicates of each treatment were mixed on three successive days. Diets were stored in glass jars in the dark at 23-26 C for 12 weeks. Duplicate 5 g samples were ground and analyzed for tocopherols on days 0, 3, 6, 9, 12, 15, 18, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84. Tocopherol analysis was done as previously described (Section 1, herein).

Data were analyzed utilizing analysis of variance and general linear model (SAS, 1982). Simple linear, semi-logarithmic linear regressions, and regression slope

[1]D,L-alpha-tocopheryl acetate, United States Biochemical Corporation, Cleveland, Ohio.

comparisons were calculated and analyzed as described by Snedecor and Cochran (1980). Biological decay constants and half lives were calculated as described by Rescigno and Segre (1966).

## Results and Discussion

Alpha-tocopheryl acetate (ATA) levels (table 2) of the NOTM diet containing no added SBO remained stable during storage. The addition of TM, Cu, Zn or Mn had no effect ($P>.1$) on the destruction of ATA. The addition of Fe resulted in an increased ($P<.05$) rate of ATA destruction. Klaui (1973) found that ATA was stable during storage in diets containing standard trace mineral mixes, but high levels of individual minerals were not tested. The results of this experiment suggest that some trace minerals may affect the stability of ATA. The addition of SBO had no effect ($P>.1$) on the destruction of ATA.

The level of alpha-tocopherol (table 3) in the NOTM diet decreased ($P<.01$) during the 84 day storage period to about 50% of the initial level. The loss of natural tocopherols in the NOTM diet during storage suggests that the natural tocopherols have a limited stability. Young et al. (1975) found that the alpha-tocopherol in mixed diets containing artificially dried corn was stable during storage. The diets used by Young et al. (1975) contained ethoxyquin, while the diets used in this experiment did not. The difference in the storage stability of the natural tocopherols may be a result

of the increased demand for a dietary antioxidant in the diets used in this experiment.

The rate of alpha-tocopherol destruction was not affected (P>.1) by the addition of TM. Richter et al. (1982) reported that the addition of trace mineral mixes to swine diets decreased the natural tocopherol levels during storage. The trace mineral mix used in this experiment seemed to exhibit little affect on alpha-tocopherol. The rate of destruction of alpha-tocopherol was increased by the addition of Cu (P<.001), Fe (P<.01), Zn (P<.05) or Mn (P<.05) to the diets. Alpha-tocopherol levels were reduced to near zero after 15 days of storage in diets containing 250 ppm added Cu. Growth promoting levels of copper are widely used in the swine industry (Ewan, 1986) and had the greatest destructive effect on the natural tocopherols. Ionized copper and iron are often included in lists of factors that increase the rate of destruction of natural tocopherols (Ullery, 1981). Waddell and Steenbock (1931) associated the presence of dietary iron with the destruction of natural tocopherols in rat diets.

The addition of SBO resulted in an increased rate of alpha-tocopherol destruction (table 3) in the TM (P<.01), Fe (P<.01), Zn (P<.01) and Mn (P<.05) supplemented diets. The increased rate of alpha-tocopherol destruction suggests that the presence of trace minerals in diets increases the peroxidation of fats in the diet increasing the need for

dietary antioxidants. The failure of added SBO to increase the rate of tocopherol destruction in the NOTM diet suggests that trace minerals are important in the mechanism of tocopherol destruction associated with added dietary fat.

Gamma-tocopherol levels (data not shown) were affected similarly to alpha-tocopherol by all mineral and SBO treatments.

The results of this experiment suggests that ATA is stable during storage and the stability of natural tocopherol is limited in diets containing NOTM. The stability of alpha-tocopheryl acetate seems to be affected by the addition of excess levels of Fe, while natural tocopherols were destroyed by excess levels of all minerals tested. These results suggest that corn-soybean meal based diets containing growth promoting levels of copper or excess levels of trace minerals may require the addition of ATA to maintain recommended levels of vitamin E during storage, especially in the presence of added dietary fat.

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Table 1. Composition of experimental diets

| Ingredient | 0% SBO diet | 1% SBO diet |
|---|---|---|
| | % | |
| Corn, yellow | 71.2 | 70.2 |
| Soybean meal, 48.5% | 24.6 | 24.6 |
| Dicalcium phosphate | 1.1 | 1.1 |
| Soybean oil | | 1.0 |
| Calcium carbonate | 0.9 | 0.9 |
| Salt | 0.3 | 0.3 |
| Mineral mix [1] | 1.0 | 1.0 |
| Vitamin mix [2] | 1.0 | 1.0 |
| Analyzed composition | | |
| Crude protein (%) | 17.5 | 17.1 |
| Gross energy (kcal/kg) | 3758 | 3837 |
| Ether extract (%) | 2.1 | 3.1 |
| Selenium (ppm) | .37 | .36 |
| Copper (ppm) | | |
| NOTM | 5.4 | 5.0 |
| TM | 9.0 | 8.7 |
| 250 ppm added | 262.8 | 252.2 |
| Iron | | |
| NOTM | 255.6 | 219.5 |
| TM | 327.7 | 312.6 |
| 1000 ppm added | 870.3 | 870.4 |
| Zinc | | |
| NOTM | 29.2 | 28.2 |
| TM | 123.5 | 110.1 |
| 1000 ppm added | 843.9 | 868.1 |
| Manganese | | |
| NOTM | 19.4 | 17.8 |
| TM | 43.8 | 37.0 |
| 100 ppm added | 110.6 | 112.2 |

[1]Contributed per kg of diet: 1.5 mg iodine, 30 or 100 mg manganese, 5 or 250 mg copper, 100 or 1000 mg iron, 100 or 1000 mg zinc. Corn was substituted for trace mineral mix in NOTM diet.

[2]Contributed per kg of diet: 4400 IU vitamin A palmitate, 1100 IU vitamin $D_2$, 6.6 mg riboflavin, 17.6 mg d-pantothenic acid, 33 mg niacin and 22 ug vitamin $B_{12}$.

Table 2. Alpha-tocopheryl acetate levels during storage (mcg/g)

| Days stored | NOTM | TM | Cu | Fe | Zn | Mn |
|---|---|---|---|---|---|---|
| | ---------- | ---------- | no added SBO | ---------- | ---------- | ---------- |
| 0 | 17.1 | 15.0 | 15.3 | 13.3 | 13.1 | 13.0 |
| 6 | 13.3 | 12.1 | 13.1 | 14.4 | 15.1 | 14.5 |
| 15 | 15.1 | 13.6 | 13.4 | 13.7 | 15.4 | 14.2 |
| 21 | 13.8 | 14.6 | 14.4 | 14.3 | 13.7 | 14.2 |
| 42 | 12.8 | 12.7 | 9.6 | 12.4 | 13.2 | 12.5 |
| 63 | 12.7 | 13.9 | 10.7 | 11.8 | 12.7 | 11.4 |
| 84 | 16.0 | 12.6 | 11.9 | 11.1 | 12.4 | 13.8 |
| Simple regression | | | | | | |
| Intercept | 14.8 | 13.9 | 13.8 | 14.2 | 14.9 | 13.6 |
| Slope | -.011 | -.012 | -.036 | -.043[a] | -.024 | -.003 |
| $r^2$ | .07 | .12 | .45 | .62 | .33 | .01 |
| Half-life (days) | 880 | 826 | 247 | 195 | 386 | 2980 |
| Days stored | ---------- | ---------- | 1% added SBO | ---------- | ---------- | ---------- |
| 0 | 16.0 | 16.4 | 13.9 | 13.4 | 15.6 | 14.9 |
| 6 | 14.2 | 13.8 | 12.9 | 12.6 | 13.5 | 13.6 |
| 15 | 13.2 | 14.6 | 13.1 | 15.4 | 14.1 | 14.5 |
| 21 | 13.8 | 13.8 | 13.6 | 13.9 | 13.3 | 13.4 |
| 42 | 11.5 | 12.3 | 11.5 | 10.4 | 13.3 | 13.0 |
| 63 | 12.7 | 13.9 | 10.4 | 9.8 | 13.0 | 12.8 |
| 84 | 11.7 | 12.7 | 12.4 | 11.3 | 12.9 | 14.2 |
| Simple regression | | | | | | |
| Intercept | 14.1 | 15.2 | 13.2 | 14.0 | 14.3 | 14.1 |
| Slope | -.025 | -.018 | -.032 | -.043 | -.015 | -.009 |
| $r^2$ | .38 | .18 | .38 | .57 | .14 | .09 |
| Half-life (days) | 360 | 569 | 260 | 195 | 628 | 1040 |

[a]Different from NOTM and TM ($P<.05$).

Table 3. Alpha-tocopherol levels during storage (mcg/g)

| Days stored | NOTM | TM | Cu[a] | Fe[b] | Zn | Mn |
|---|---|---|---|---|---|---|
| | ---------- no added SBO ---------- | | | | | |
| 0 | 5.25 | 4.24 | 3.81 | 3.74 | 4.46 | 4.48 |
| 6 | 3.90 | 3.29 | 1.96 | 3.22 | 4.14 | 4.00 |
| 15 | 3.90 | 3.61 | .04 | 2.23 | 3.84 | 3.55 |
| 21 | 3.77 | 3.48 | | 1.82 | 2.94 | 3.22 |
| 28 | 3.34 | 2.71 | | 1.36 | 2.86 | 2.88 |
| 42 | 3.39 | 3.01 | | 1.17 | 2.45 | 2.30 |
| 63 | 2.67 | 1.86 | | 1.12 | 2.00 | 2.35 |
| 84 | 1.89 | 2.23 | | 1.24 | 1.52 | 1.81 |
| Semi-logarithmic linear regressions antilog$_{10}$ | | | | | | |
| intercept | 3.35 | 2.92 | .79 | 2.35 | 2.81 | 2.92 |
| B value | .991 | .993 | .752[c] | .964[d] | .989[e] | .989[e] |
| $r^2$ | .86 | .76 | .87 | .96 | .95 | .92 |
| Half-life (days) | 84.7 | 92.2 | 2.4 | 19.0 | 58.2 | 61.5 |
| Days stored | ---------- 1% added SBO ---------- | | | | | |
| 0 | 5.19 | 5.09 | 5.36 | 4.13 | 5.32 | 5.50 |
| 6 | 5.04 | 4.07 | 1.94 | 3.86 | 4.60 | 3.73 |
| 15 | 4.78 | 3.44 | .15 | 1.37 | 4.09 | 3.57 |
| 21 | 4.79 | 2.92 | | .93 | 3.03 | 4.02 |
| 28 | 4.07 | 3.23 | | .64 | 3.09 | 3.64 |
| 42 | 3.63 | 2.88 | | | 1.91 | 2.33 |
| 63 | 2.84 | 1.90 | | | 1.57 | 1.62 |
| 84 | 2.52 | 1.62 | | | 1.26 | 1.75 |

| Semi-logarithmic linear regressions antilog$_{10}$ | | | | | | |
|---|---|---|---|---|---|---|
| intercept | 3.85 | 2.88 | 1.30 | 1.21 | 2.58 | 2.99 |
| B value | .991 | .986[f] | .796 | .927[f] | .979[f] | .986[g] |
| $r^2$ | .93 | .92 | .89 | .96 | .96 | .92 |
| Half-life (days) | 78.3 | 52.6 | 3.1 | 9.0 | 35.4 | 46.7 |

[a]15 day regression line was used for Cu.

[b]28 day regression line was used for Fe.

[c]Different from NOTM and TM (P<.001).

[d]Different from NOTM and TM (P<.01).

[e]Different from NOTM and TM (P<.05).

[f]Diet with SBO different from diet without SBO (P<.01).

[g]Diet with SBO different from diet without SBO (P<.05).

## SECTION 3. THE EFFECT OF TRACE MINERAL ADDITION AND FEED STORAGE ON THE STABILITY OF VITAMIN E AND THE PERFORMANCE OF GROWING PIGS

### Abstract

A 13 week trial was conducted using 32 pigs to determine the effects of dietary copper (Cu, 250 ppm) and alpha-tocopheryl acetate (ATA, 22 IU/kg) on the performance, serum enzymes, serum and tissue tocopherols and antibody production in growing pigs. Pigs were fed corn-soybean meal diets containing 21% crude protein (CP) the first 4 weeks and 18% CP the remainder of the trial. All feed was stored a minimum of 14 days prior to use. The addition of Cu decreased ($P<.01$) the level of natural tocopherols in the feed. Alpha-tocopherol levels were less than 1 mcg/g in the starter diet and 2 mcg/g in the grower diet after 14 days of storage. ATA levels were reduced ($P<.05$) in the starter diet by the addition of Cu. ATA levels were not affected ($P>.1$) by Cu in the grower diet. The addition of Cu to diets containing no ATA improved average daily gain and average daily feed intake during weeks 5 to 13, resulting in a Cu X ATA interaction ($P<.05$). The addition of Cu or ATA had no ($P>.1$) effect on serum glutathione peroxidase or lactic dehydrogenase. Serum tocopherols were reduced ($P<.05$) by the addition of Cu during the first 4 weeks and increased ($P<.05$) by ATA addition during the entire experiment. The addition of ATA increased ($P<.05$) the tocopherol levels in ham, heart,

pancreas, kidney and kidney fat, but had no effect (P>.1) on the tocopherol levels of the adrenal glands, lymph nodes, lung, spleen, liver, psoas or longissimus muscles, backfat and bile. Antibody production in response to immunization with sheep red blood cells was not affected (P>.1) by the addition of Cu or ATA. The addition of ATA to the diets of growing pigs had no effect on performance or serum enzymes, but did improve tocopherol status. The addition of Cu had no effect on performance, serum enzymes or tissue tocopherol levels or antibody production.

Key Words: Swine, tocopherols, copper.

## Introduction

The addition of growth promoting levels of copper to swine diets is a common practice among swine producers (Ewan, 1986). Dove and Ewan (1987b) reported that the addition of copper to swine grower diets increased the destruction of alpha-tocopherol. The addition of excess levels of a trace mineral mix has also been reported to increase the destruction of tocopherols (Richter et al., 1982).

The NCR-42 Committee on Swine Nutrition (1974) found that the addition of Cu or alpha-tocopheryl acetate to swine diets resulted in a variable performance response. No interaction between vitamin E and Cu was demonstrated. This experiment was designed to study the effects of diets deficient in vitamin E due to Cu addition on the performance,

serum enzymes, serum and tissue tocopherol levels and antibody production of growing pigs.

## Materials and Methods

Thirty-two, crossbred pigs (Landrace X Yorkshire X Duroc, average initial weight 4.7 kg) were assigned from litter outcome group to one of four diets. Pigs were placed on experimental diets at weaning and remained on the same dietary treatment for the duration of the thirteen week experiment. Two levels of copper, as anhydrous cupric sulfate, (5 or 250 ppm) and two levels of alpha-tocopheryl acetate[1] (0 or 22 IU/kg) were used in a 2 X 2 factorial arrangement of treatments.

Pigs were housed in individual pens for the first 4 weeks of the experiment and 2/pen for the remainder of the experiment. Pigs had ad libitum access to feed and water. Pigs were weighed and feed consumption was determined weekly. Weekly serum samples were stored at -20 C until tocopherol levels and lactic dehydrogenase (LDH) and glutathione peroxidase (GSH-Px) activities were determined. Serum enzyme activities were determined within 24 hours after blood samples were collected.

Two pigs from each treatment were slaughtered at the conclusion of the 13 week experiment. Samples were taken from the adrenal gland, lymph nodes, ham, heart, lung,

[1]D,L-Alpha-tocopheryl acetate, United States Biochemical Corporation, Cleveland, Ohio.

pancreas, spleen, kidney, kidney fat, backfat, bile, psoas muscle, longissimus muscle, left and right medial liver and left and right lateral liver. Tissue samples were frozen in liquid N within one-half hour after the animal was slaughtered and stored at -20 C until analyzed for tocopherols.

The starter diet was fed during the first four weeks and the grower diet was fed during the remainder of the experiment (table 1). Diets were formulated to meet or exceed all NRC (1979) recommendations except for vitamin E and selenium. No supplemental selenium was added to the diets. All diets were stored for at least two weeks prior to use in the experiemnt. Feed samples were collected weekly and stored at -20 C until analyzed for tocopherols.

Serum LDH was determined by the ultraviolet spectrophotometric method of Amador et al. (1963). Serum GSH-Px was determined by the ultraviolet spectrophotometric method of Paglia and Valentine (1967). Serum and feed tocopherols were determined by a modified fluorometric HPLC method of Cort et al. (1983) as previously described (Section 1, herein). Tissue tocopherols were determined by homogenizing 1.0 g of tissue in 10 ml (w/v) phosphate-EDTA buffer (pH 7.0) and extracting with hexane as described for serum tocopherols (Section 1, herein). Selenium was determined by the method of Olson et al. (1975).

All pigs were injected (5 ml IV) with a 40% (V/V) solution of sheep red blood cells (SRBC) in sterile phosphate buffer on weeks 2, 5, 8 and 11. Blood collected at weeks 3, 6, 9 and 12 was analyzed for serum hemoglutination titer against SRBC (Buschman et al., 1974). Titers were calculated as the $\log_2$ of the highest dilution exhibiting agglutination.

All data were analyzed by the general linear model (SAS, 1982).

## Results and Discussion

Feed tocopherols. Alpha-tocopheryl acetate (ATA) levels were decreased ($P<.05$) by the addition of Cu in the starter diet (table 2). The ATA level in the grower diet was not affected ($P>.1$) by the addition of Cu (table 3). Dove and Ewan (1987b) found that the addition of Cu had no effect on the rate of alpha-tocopheryl acetate destruction. The different levels of dietary fat and difference in storage enviroment during this experiment may account for the differences in the destructive effect of Cu on alpha-tocopheryl acetate in feeds.

Alpha-tocopherol levels were not affected by the addition of ATA ($P>.1$), but were decreased ($P<.01$) by the addition of Cu (table 2 and 3). After 14 days of storage the alpha-tocopherol levels of the Cu added diets were near 0 mcg/g for the starter diets and less than 2 mcg/g for the grower diet. The added fat in the starter diet probably accounts for the differences in the tocopherol levels between

the starter and grower diets. The diet containing added Cu but no added ATA was considered to be tocopherol deficient. The effect of Cu on the natural tocopherols is similar to the results of Dove and Ewan (1987a, b).

Performance. The addition of Cu or ATA had no effect ($P>.1$) on the average daily gain (ADG), average daily feed intake (ADF) or the gain:feed ratio during weeks one to four (table 4). The addition of Cu to diets containing no ATA increased ADG and ADF during weeks 5 to 13, resulting in a Cu by ATA interaction ($P<.05$). The growth promoting effects of Cu are variable and appear to be influenced by environmental, management and genetic differences (NCR-42, 1974). The performance data of this experiment suggests that diet composition may affect the growth promoting ability of Cu.

Serum enzymes. Serum glutathione peroxidase (GSH-Px) and lactic dehydrogenase (LDH) activities were not affected ($P>.1$) by the addition of Cu or ATA (table 5). Serum GSH-Px activity increased ($P<.05$) during the first 8 weeks and then remained stable. GSH-Px is a selenium dependent enzyme (Rotruck et al., 1973) and the increased GSH-Px activity during the experiment indicates that the pigs were receiving adequate selenium.

Serum LDH activity decreased ($P<.05$) during the first week of the experiment and remained stable through out the rest of the experiment. Nutritional muscular dystrophy (NMD) is indicated by elevated serum LDH activity (Paulson et al.,

1968) and is associated with vitamin E/selenium deficiencies (Hartley and Grant, 1961). Serum LDH activity increases in pigs with vitamin E/selenium deficiencies. Although the pigs receiving the diet supplemented with Cu and with no added ATA received little vitamin E, the stability of the LDH activity suggests that no vitamin E/selenium deficiency was present.

Serum tocopherols. Serum alpha-tocopherols decreased ($P<.05$) during the first week of the experiment (table 5). This is consistent with previous reports (Dove and Ewan, 1987a; Meyer et al., 1981). Serum alpha-tocopherol levels were increased ($P<.05$) by the addition of ATA to the diet. Meyer et al. (1981) reported that serum tocopherol increases linearly with increasing levels of supplemental ATA.

Serum tocopherol levels were decreased ($P<.05$) by the addition of Cu to the diet during the first 4 weeks of the experiemnt. The serum tocopherol level of the pigs receiving 250 ppm Cu increased after the diet change, but never returned to the level of the pigs receiving 5 ppm Cu. These results are similar to previously reported results (Dove and Ewan, 1987a).

Tissue tocopherols. A numerical difference in the tocopherol levels was observed for all tissues as a result of the addition of both Cu and ATA (table 6). Because tissue tocopherol levels were highly variable and the small number of animals sampled ATA addition resulted in increases ($P<.05$) in the tocopherol levels of ham, heart, pancreas, kidney,

right lateral lobe of the liver and kidney fat. The tocopherol levels of the heart, pancreas and liver seem to best reflect the level of tocopherol intake.

Response to SRBC. Antibody production against SRBC was not influenced by the addition of Cu or ATA to the diet (table 7). Bendich et al. (1986) found that the dietary vitamin E requirement needed to optimize certain immune functions in the rat were higher than the requirement for optimum performance. The response in antibody production in this experiment is similar to results of Kornegay et al. (1986), who found that 55 IU/kg added vitamin E did not stimulate immune responses to lysozymes or SRBC in weanling pigs. The results of this experiment also suggest that low dietary levels of vitamin E do not adversely affect antibody production against SRBC.

The performance, serum enzyme activities and antibody production of pigs receiving diets low in tocopherol were not affected, suggesting that pigs are able to maintain tocopherol status for periods exceeding three months in the presence of recommended levels of Se. The increase in serum and tissue tocopherols with supplementation suggests that while pigs were maintaining adequate tocopherol levels, supplementation was needed to maintain optimal serum and tissue tocopherol levels.

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Table 1. Composition of experimental diets

| Ingredient | Starter | Grower |
|---|---|---|
| | % | |
| Corn, ground yellow | 48.6 | 70.9 |
| Soybean meal, 48% | 29.0 | 24.6 |
| Whey | 15.0 | |
| Soybean oil, crude | 2.9 | |
| Dicalcium phosphate | 1.0 | 1.1 |
| Calcium carbonate | 1.0 | .9 |
| Iodized salt | .25 | .25 |
| Antibiotic [a] | .25 | .25 |
| Mineral mix [b] | 1.0 | 1.0 |
| Vitamin mix [c] | 1.0 | 1.0 |
| Analyzed composition | | |
| Crude protein (%) | 20.4 | 18.2 |
| Gross energy (kcal/kg) | 4129 | 3950 |
| Selenium (ppm) | .33 | .36 |
| Copper (ppm) | | |
| 5 ppm diet | 12 | 10 |
| 250 ppm added diet | 251 | 273 |
| Calculated composition | | |
| Lysine (%) | 1.20 | .95 |

[a]Contributed per kg of diet: 110 mg sulfamethazine, 55 mg penicillin and 110 mg chlortetracycline.

[b]Contributed per kg of diet: 30 mg Mn, 1.5 mg I, 5 or 250 mg Cu, 100 mg Fe and 100 mg Zn.

[c]Contributed per kg of diet: 4400 IU vitamin A palmitate, 1100 IU vitamin $D_2$, 6.6 mg riboflavin, 17.6 mg d-pantothenic acid, 33 mg niacin, 22 ug vitamin $B_{12}$, 4.4 mg ethoxyquin and 0 or 22 IU alpha-tocopheryl acetate.

Table 2. Least squares means of starter diet tocopherol content during storage (mcg/g)

| | ppm Copper | | | |
|---|---|---|---|---|
| | 5 | 5 | 250 | 250 |
| | IU alpha-tocopheryl acetate | | | |
| Days Stored | 0 | 22 | 0 | 22 |
| | Alpha-tocopheryl acetate | | | |
| 0 | 1.19 | 12.75 | 1.41[a] | 12.82[a] |
| 14 | 1.54 | 13.36 | 1.41 | 13.97 |
| 28 | 1.46 | 13.68 | 1.19 | 10.79 |
| 42 | 1.66 | 15.97 | 1.17 | 8.34 |
| | Alpha-tocopherol | | | |
| 0 | 4.38 | 4.24 | 2.64[a] | 3.21[a] |
| 14 | 3.52 | 3.08 | 0.00 | 0.00 |
| 28 | 3.15 | 2.96 | 0.00 | 0.00 |
| 42 | 3.66 | 3.67 | 0.00 | 0.00 |

[a]Copper effect ($P<.01$).

Table 3. Least squares means of the grower diet tocopherol content during storage (mcg/g)

| | ppm Copper | | | |
|---|---|---|---|---|
| | 5 | 5 | 250 | 250 |
| | IU alpha-tocopheryl acetate | | | |
| Days Stored | 0 | 22 | 0 | 22 |
| | Alpha-tocopheryl acetate | | | |
| 1 | 1.33 | 15.42 | 1.19 | 17.04 |
| 15 | 1.44 | 13.67 | 1.46 | 18.09 |
| 29 | 1.18 | 13.26 | 1.15 | 13.44 |
| 43 | 1.76 | 12.67 | 0.97 | 6.33 |
| 50 | 1.59 | 12.48 | 1.28 | 12.85 |
| | Alpha-tocopherol | | | |
| 1 | 4.72 | 4.71 | 2.99[a] | 2.62[a] |
| 15 | 4.28 | 4.03 | 1.77 | 1.03 |
| 29 | 3.76 | 3.89 | 0.44 | 0.08 |
| 43 | 3.36 | 1.91 | 0.06 | 1.68 |
| 50 | 4.05 | 3.92 | 0.70 | 0.80 |

[a]Copper effect ($P<.01$).

Table 4. Least square means of performance during copper and tocopherol addition to the diets of growing pigs

| | ppm Copper | | | |
|---|---|---|---|---|
| | 5 | 5 | 250 | 250 |
| | IU alpha-tocopheryl acetate | | | |
| Period | 0 | 22 | 0 | 22 |
| | Average Daily Gain (kg/d) | | | |
| Weeks 1 to 4 [a] [b] | 0.40 | 0.38 | 0.39 | 0.31 |
| Weeks 5 to 13 [b] | 0.77 | 0.75 | 0.84 | 0.75 |
| | Average Daily Intake (kg/d) | | | |
| Weeks 1 to 4 [b] | 0.60 | 0.60 | 0.61 | 0.55 |
| Weeks 5 to 13 | 2.02 | 2.05 | 2.30 | 1.86 |
| | Gain:Feed | | | |
| Weeks 1 to 4 | 0.67 | 0.65 | 0.63 | 0.54 |
| Weeks 5 to 13 | 0.38 | 0.37 | 0.37 | 0.40 |

[a] ATA effect ($P<.05$).

[b] Cu * ATA interaction ($P<.05$).

Table 5. Least square means of serum enzymes and serum alpha-tocopherol of pigs fed added dietary copper and alpha-tocopheryl acetate.

| Week | ppm Copper: 5 | 5 | 250 | 250 |
|---|---|---|---|---|
| | IU alpha-tocopheryl acetate: 0 | 22 | 0 | 22 |
| | LDH (units/ml) | | | |
| 0 | 432 | 418 | 408 | 402 |
| 1 | 298[a] | 298[a] | 296[a] | 316[a] |
| 4 | 307 | 332 | 341 | 342 |
| 13 | 293 | 243 | 263 | 235 |
| | GSH-Px (units/ml)[b] | | | |
| 0 | 0.7 | 0.7 | 0.7 | 0.7 |
| 1 | 0.8 | 0.9 | 0.7 | 0.7 |
| 4 | 0.9 | 0.9 | 1.0 | 0.8 |
| 8 | 1.3 | 1.3 | 1.3 | 1.2 |
| 13 | 1.4 | 1.2 | 1.5 | 1.3 |
| | Alpha-tocopherol (mcg/ml) | | | |
| 0 | 0.28 | 0.31 | 0.31 | 0.35 |
| 1 c d | 0.09 | 0.18 | 0.06 | 0.10 |
| 4 c e | 0.17 | 0.49 | 0.03 | 0.07 |
| 8 c d | 0.18 | 0.68 | 0.11 | 0.59 |
| 13 c d | 0.20 | 0.71 | 0.14 | 0.64 |

[a]Decreased from initial (P<.05).

[b]Increased with time (P<.05).

[c]Cu effect (P<.05).

[d]ATA effect (P<.05).

[e]Cu * ATA interaction (P<.05).

Table 6. Least squares means of tissue alpha-tocopherol levels of pigs fed added dietary copper and alpha-tocopheryl acetate (mcg/ml or g)

| | ppm Copper | | | | |
|---|---|---|---|---|---|
| | 5 | 5 | 250 | 250 | |
| | IU alpha-tocopheryl acetate | | | | |
| Tissue | 0 | 22 | 0 | 22 | SE[a] |
| Adrenal gland | 1.20 | 4.44 | 0.85 | 5.78 | 2.35 |
| Bile[b] | 0.45 | 1.53 | 0.18 | 1.01 | .60 |
| Ham[b] | 0.31 | 1.75 | 0.26 | 1.37 | .75 |
| Heart[b] | 1.02 | 4.07 | 0.52 | 2.88 | 1.59 |
| Kidney[b] | 0.67 | 1.33 | 0.22 | 1.36 | .65 |
| Liver | | | | | |
| Left lateral lobe | 0.61 | 3.14 | 0.49 | 2.84 | 1.26 |
| Left medial lobe | 0.65 | 3.15 | 0.58 | 2.86 | 1.38 |
| Right medial lobe[b] | 0.63 | 3.34 | 0.50 | 2.64 | 1.17 |
| Right lateral lobe[b] | 0.55 | 3.27 | 0.42 | 2.77 | 1.48 |
| Average (all lobes) | 0.61 | 3.23 | 0.50 | 2.78 | |
| Longissimus Dorsi | 0.52 | 1.02 | 0.15 | 0.87 | .55 |
| Lung | 0.25 | 1.81 | 0.21 | 1.51 | 1.00 |
| Lymph nodes[b] | 0.90 | 3.41 | 1.48 | 3.09 | 1.29 |
| Pancreas | 0.81 | 5.52 | 0.54 | 3.53 | 1.24 |
| Psoas Muscle | 0.24 | 1.65 | 0.16 | 1.43 | .73 |
| Spleen | 0.59 | 3.19 | 0.51 | 2.68 | 1.45 |
| Kidney Fat[b] | 0.28 | 1.31 | 0.28 | 1.18 | .69 |
| Back Fat | 0.10 | 0.95 | 0.19 | 0.57 | .42 |

[a]Standard Error of the mean.

[b]ATA effect ($P<.05$).

Table 7. Antibody titers against SRBC ($log_2$ dilution)

| | ppm Copper | | | | |
|---|---|---|---|---|---|
| | 5 | 5 | 250 | 250 | |
| | IU alpha-tocopheryl acetate | | | | |
| Week | 0 | 22 | 0 | 22 | SE[a] |
| 3 | 1.4 | 1.6 | 0.9 | 0.8 | .26 |
| 6 | 3.1 | 4.1 | 3.4 | 2.6 | .40 |
| 9 | 3.4 | 3.9 | 3.4 | 2.6 | .43 |
| 12 | 4.3 | 3.5 | 3.4 | 2.6 | .33 |

[a]Standard error of the mean.

## SUMMARY AND DISCUSSION

The destructive effect of trace minerals on tocopherols has been known since Waddell and Steenbock (1931) reported the destruction of tocopherols in rat diets containing ferric chloride. Since then ionizing trace minerals, especially iron and copper, have been considered to contribute to the loss of tocopherol activity in feeds (Ullrey, 1981). However, the rate of tocopherol destruction caused by individual trace minerals has not been quantified. The effect of trace minerals on the tocopherol status of the feed was of particular importance because high levels of trace minerals have been found in swine diets fed in Iowa (Ewan, 1986).

The alpha-tocopherol level of corn-soybean meal diets can be considered marginally adequate under most conditions. Vitamin E/selenium deficiencies continue to be reported by producers and veterinarians in pigs fed diets calculated to contain recommended amounts of vitamin E and selenium. The conditions or factors causing the field deficiencies have been difficult to duplicate experimentally.

Vitamin E deficiency symptoms were not produced under the conditions used in these experiments. Pigs fed diets containing very low levels of tocopherols and adequate selenium performed as well as pigs fed supplemental alpha-tocopheryl acetate. There were also no differences in serum enzymes or immune response to sheep red blood cells.

This indicates that pigs are able to maintain minimum tocopherol levels for periods greater than 13 weeks when fed diets containing very low levels of tocopherols in the presence of adequate selenium. However, serum or tissue tocopherol levels of pigs fed vitamin E deficient diets were decreased, indicating that pigs were not receiving adequate tocopherols to maintain serum or tissue levels. The long term effects of low tissue tocopherols levels are not known, but increased peroxidation of the tissue could be possible. There were no reports of vitamin E deficiencies in the presence of adequate selenium found in the literature. This may indicate that vitamin E is required by the pig only when dietary selenium levels are low. The selenium levels (.33 to .36 ppm) in the diets used in these experiments were above the recommended levels, even though no supplemental selenium was added to any of the diets.

The alpha-tocopheryl acetate was found to be stable in the presence of 1% soybean oil or any of the minerals tested, except iron. The analysis performed in these experiments did not determine how iron increased the rate of alpha-tocopheryl destruction. The relative stability of alpha-tocopheryl acetate indicates that diets supplemented with alpha-tocopheryl acetate should be able to meet the pig's need for tocopherol after a storage period of up to 3 months and probably longer.

Alpha-tocopherol levels decreased during storage. A half life of 85 days was found for diets containing no trace minerals or soybean oil. The initial alpha-tocopherol level in the diet used in the storage experiment was about 1/2 of the 11 IU/kg of tocopherol recommended by NRC (1979) and about 1/4 of the reported NRC 1988 (Dr. V. Speer, Chairman of NRC 1988 committee, personal communication) recommendation. Losses during prolonged storage would cause this diet to be deficient in vitamin E.

The addition of 1% crude soybean oil had no effect on the rate of alpha-tocopherol destruction in the diet that contained no trace minerals, but increased the rate of alpha-tocopherol destruction in diets containing minerals. It appears that a mineral by fat interaction is responsible for the effect on the rate of alpha-tocopherol destruction. It is difficult to determine if the minerals are catalyzing the peroxidation of the fat or if the fat is supplying a substrate for the direct destruction of alpha-tocopherol by the minerals. Tocopherol levels in swine diets containing fat may need further investigation if fats continue to gain popularity as energy sources in swine diets.

The addition of growth promoting levels of copper (250 ppm) increased the rate of alpha-tocopherol destruction and decreased the half life to 2.4 days. The addition of 250 ppm copper resulted in extremely low alpha-tocopherol levels after 15 days of storage and supplementation of these diets

with alpha-tocopheryl acetate should be recommended. In these experiments diets that had been stored 15 days prior to use and contained 250 ppm copper failed to produce vitamin E deficiencies during a 13 week feeding trial, but serum and tissue tocopherols tended to be decreased. This may indicate that the pig is able to store adequate vitamin E to maintain tocopherol status for periods longer than 13 weeks or that other environmental factors are required for pigs to develop vitamin E deficiencies.

Diets containing excess levels of iron (1000 ppm), zinc (1000 ppm) or manganese (100 ppm) increased the rate of alpha-tocopherol destruction during storage when compared to the diets containing no trace minerals or a standard trace mineral mix and seemed to have the largest effect on the increased destruction of alpha-tocopherol due to crude soybean oil addition. The combination of 1000 ppm iron and soybean oil increased the rate of alpha-tocopherol destruction and decreased alpha-tocopherol levels to near zero after 28 days of storage. Supplemental alpha-tocopheryl acetate may be needed in diets containing excess trace minerals, especially in the presence of fats, to maintain optimal tocopherol status.

In the experiments reported in this dissertation, diets were changed, to decrease protein and energy content, after four weeks as recommended by NRC (1979). The removal of the fat from the diet at the end of four weeks was probably

responsible for the changes in the serum tocopherol levels during weeks four and five seen in these experiments. These experiments were conducted to follow practical management techniques as closely as possible in an effort to duplicate the vitamin E deficiencies reported in commercial swine herds. The change in diet at the end of 4 weeks appeared to affect the tocopherol status of the pigs in 2 of 3 performance experiments. This may indicate that the tocopherols in the starter diet were less available to the pigs or that the pigs were unable to efficiently use the dietary tocopherols for a 3 to 4 week period following weaning. Additional research into the utilization of and digestibility of tocopherols by weanling pigs is needed.

The experiments reported in this dissertation have shown that alpha-tocopheryl acetate is stable under most conditions, but not all and that alpha-tocopherol is very unstable. Future research should investigate the effects of temperature and humidity on the stability of both alpha-tocopheryl acetate and alpha-tocopherol. Experimental attempts to produce a vitamin E deficiency in pigs fed diets adequate in selenium should be continued. Diets containing 250 ppm copper with 2 to 5% crude soybean oil could be used. Diets would need to be stored prior to use and if possible a single batch of feed should be mixed to be used through out the entire experiment.

Several other factors have been suggested as possible contributing factors in the vitamin E deficiencies reported from commercial swine producers. Among these are viral diseases, mycotoxins, stress, genetics and management techniques. All of these factors deserve additional investigation.

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